BIOCHEMICAL GENETIC STUDIES ON THE OIL SARDINE, *Sardinella longiceps* (CUVIER AND VALENCIENNES, 1847) FROM SELECTED CENTRES OF THE WEST COAST OF INDIA

THESIS SUBMITTED IN PARTIAL FULFILMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN MARINE SCIENCE OF THE
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
KOCHI - 682 022

BY

P. VENKITA KRISHNAN

INDIAN COUNCIL OF AGRICULTURAL RESEARCH
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
P. B. No. 1603, KOCHI - 682 014
INDIA

DECEMBER 1993

Dedicated
to
MY PARENTS

DECLARATION

I hereby declare that this thesis entitled **BIOCHEMICAL GENETIC STUDIES ON THE OIL SARDINE, SARDINELLA LONGICEPS (CUVIER AND VALENCIENNES, 1847) FROM SELECTED CENTRES OF THE WEST COAST OF INDIA** has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

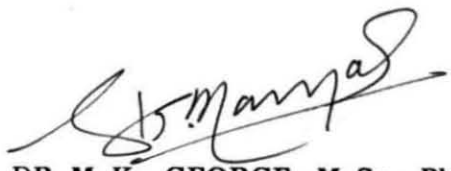
Kochi-682014

December, 1993

P. VENKITA KRISHNAN

CERTIFICATE

This is to certify that the thesis entitled **BIOCHEMICAL GENETIC STUDIES ON THE OIL SARDINE, SARDINELLA LONGICEPS (CUVIER AND VALENCIENNES, 1847) FROM SELECTED CENTRES OF THE WEST COAST OF INDIA** is a bonafide record of the work carried out by **Mr. P. VENKITA KRISHNAN** under my guidance and supervision and that no part thereof has been presented for the award of any other degree.



DR. M. K. GEORGE, M. Sc., Ph. D.
Senior Scientist
Central Marine Fisheries Research Institute
Kochi - 14.

Kochi - 682014

December, 1993

ACKNOWLEDGEMENTS

I sincerely and most profoundly record my deepest gratitude with great pleasure to Dr. M.K. George, Senior Scientist, Central Marine Fisheries Research Institute, Cochin-14 for suggesting the research problem, providing able guidance and supervising the work. I would like to extend my sincere thanks to Dr. P.S.B.R. James, Director, C.M.F.R.I., for providing all facilities needed for this investigation and to Cochin University of Science and Technology for granting me the Ph.D. registration.

I am grateful to Dr. George John, Director, D.B.T., New Delhi, my previous supervisor, for his guidance and encouragements. I also thank Dr. A.G. Ponniah, Senior Scientist, N.B.F.G.R., Allahabad for his valuable help and suggestions.

My thanks are due to Dr. I.D. Gupta, Dr. N.K. Verma and Mr.Vijayagopal, Scientists, C.M.F.R.I., for all encouragements and help during the investigation period. I also thank Dr. C. Suseelan, Senior Scientist, C.M.F.R.I., for providing me the facilities for the photographic work.

I am thankful to Mr. E.V. Radhakrishnan, Dr. N. Neelakanta Pillai, Mr. K.K. Sukumaran, Dr. P.P. Pillai and Dr. Kuriakose, Scientists, C.M.F.R.I., for helping me during specimen collection.

I gratefully acknowledge the valuable suggestion and timely help extended to me by Dr. Jose Stephan, subject expert, C.I.F.T., Dr. N. Unnikrishnan Nair, Senior Scientist, C.I.F.T., Dr. N.R. Menon, Dean of Marine

Science, Cochin University, Dr. P.V. Ramachandran Nair, Retd. Scientist, C.M.F.R.I., Dr. J. Bojan, Project Director, M.P.E.D.A., Vallarpadam and Late Mr. Sadananda Rao and Late Mr. C.P. Ramamritham, C.M.F.R.I., Kochi.

I express my deep sense of gratitude and respect to Mr. T.R. Rajamani, Senior Scientist, C.E.C.R.I., Kalamassery and Dr. L. Krishnan, Senior Scientist, C.I.B.A., Narakkal for their valuable advice and constant encouragements from the very beginning of this research work. I also wish to acknowledge here, Mr. K.L.V. Narayanan, Export Manager, M/s. Anna Aluminium Co., Cochin who helped me in various ways.

I would like to show my sincere gratitude to Mr. P. Venkita Narayanan, Lecturer, Ship Technology, C.U.S.A.T., for the computer software support.

I take this opportunity to show my indebtedness to Miss. B. Santh Begum, Research Scholar, C.M.F.R.I., Kochi for her untiring help and continuous encouragements rendered during the period of this study and especially during the compilation of reference part of this thesis.

I would like to extend my special thanks to Mr. K. Diwakar, Laboratory Assistant, ICAR-Adhoc Project, now Technical Assistant, in C.M.F.R.I., Vizakhapatnam for his continuous assistance during the investigation. I would also like to thank Mr. K.L.K. Kesavan, Artist and Mr. P. Raghavan, Photographer, C.M.F.R.I., for their timely help. My sincere thanks are due to C.M.F.R.I., Library Staff members for helping me with the library facilities

and to C.M.F.R.I., Vehicle drivers for their help during the collection of specimens.

I express my strong feeling of gratitude to some of my colleagues and friends, Mr. Tomy Prince, Mr. N.N. Mohandas, Dr. S. Vijayakumar, Dr. Paramananda Das, Dr. K.K. Vijayan, Mr. C.P. Balasubramanian, Mr.P.S. Sudheesh, Mr. S. Kandan, Mr. V. Mohan, Mr. K. Madhu and Mr. K.K. Joe for various help rendered to me during the research work.

I am very much grateful to Mrs. Sajitha, C.M.F.R.I., and Mrs. Aisha Suresh, Coastal Impex for typing the thesis, Mr. M.K. Sasi, Coastal Impex and Mr. John, Canon Xerox for xeroxing the thesis.

Above all I am very much indebted to Indian Council of Agricultural Research for awarding me the research fellowship, under the ICAR-Adhoc Scheme, "Population genetic studies on oil sardine, Sardinella longiceps to identify distinct genetic stocks".

CONTENTS

PAGE

PREFACE

The marine fish production of India was 2.29 million tonnes in 1992. The oil sardine, Sardinella longiceps alone contributed 104000 tonnes forming 4.54% of the above total marine fish production. An historic characteristic of oil sardine fishery of India is significant fluctuation in its abundance through the past few decades. During 1961-68, its catch composition ranged from 9.71 to 33.8% of total marine fish landings of India, giving an annual average of 210376 tonnes whereas it was suddenly dropped to 165586 tonnes during 1969-78 period.

Some of the general factors such as diatom production, fishing of juveniles, periodical migration, heavy natural mortality and lack of average rainfall etc. have been suspected as playing a role in the final production rate of oil sardine fishery in India. On the other hand, the collapse of Pacific sardine, Sardinops sagax was attributed to the gradual breakdown of genetically different populations of the species. Hence it is of great significance to get an insight into the genetic composition of S. longiceps of India. The main objective of the present investigation therefore, was to study the biochemical genetic variability within the species and genetic structure of its regional populations from west coast. Realising the recent report of occurrence of oil sardine fishery in east coast of India, population samples from Mandapam and Madras were also included in the present investigation. The original data gathered on the population genetics of the species have helped to interpret and evaluate the results objectively. The important conclusions drawn from a detailed discussions on the subject would throw some light on the probable process of problematic fluctuations in the abundance of oil sardine

fishery of India. The academic and applied values of present discoveries need not be emphasised. The data used for the doctoral thesis were generated during the ICAR Ad-hoc project on the "Population genetic studies on oil sardine, Sardinella longiceps to identity distinct genetic stocks", carried out at CMFRI, Cochin during the years, 1988-1991.

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

LIST OF ABBREVIATIONS USED

Enzymes:

ADH	=	Alcohol Dehydrogenase
AO	=	Aldehyde Oxidase
EST	=	Esterase
G6PD	=	Glucose-6-Phosphate Dehydrogenase
GDH	=	Glutamate Dehydrogenase
IDH	=	Isocitrate Dehydrogenase
LDH	=	Lactate Dehydrogenase
MDH	=	Malate Dehydrogenase
XDH	=	Xanthine Dehydrogenase

Alleles:

S	=	Slow
F	=	Fast
SF	=	Combination of slow and fast (Heterozygote)

Populations:

CHN	=	Cochin
CCT	=	Calicut
MRE	=	Mangalore
MDM	=	Mandapam
MAS	=	Madras

Others:

OBS	=	Observed
EXP	=	Expected
CHI	=	Chi Square
FIG	=	Figure
PLT	=	Plate
TBL	=	Table
D.D.W	=	Double Distilled Water
X	=	Intensity
IX	=	Light intensity
2X	=	Medium intensity
3X	=	Dark intensity

1. INTRODUCTION

Fishes have always been an important source of food for mankind. Naturally, man is much concerned with not only its exploitation but also its various research aspects such as taxonomy, biology, fishery, aquaculture and even genetics. Taxonomically, the unit of fishery resources is the species, a concept developed during the classical periods of Linnaeus, Lamark and Darwin. The species concept still remains as the corner stone of scientific research in various aspects of biology. Nevertheless, according to the Darwin's theory of origin of species and modern theories of evolution, the species cannot remain as a constant entity. Each species may undergo further evolutionary changes leading to further speciation. The process and order of such further speciation is primarily by reproductive isolation and transformation of its populations into different races or sub-species and later into newspecies in an unspecified evolutionary time scale.

The mathematical theories of Wagner, Jordan, Densch, Mayr etc. tried to explain how natural selection of inherent variations within the species can lead to differentiation of populations into races or sub-species (Dobzhansky, 1967, 1970; Mangaly, 1974).

The concept of species and sub-species is taken seriously by taxonomists and biologists throughout the world for practical and applied purposes. Since species and sub-species concepts have special implication in exploitation and conservation of fishery resources, fishery biologists have always been specially interested in searching and identifying stocks/races/sub-species of many economically important fish species. Classical racial investigations on herring populations was conducted by Heincke as early as 1898.

only genotype data not directly influenced by environmental parameters are considered (Moller, 1971; Jamieson, 1974). According to the second definition the homogeneity arises when populations show similar gene frequencies or a common genepool whereas heterogeneity will be implied when significant differences between populations occur at genefrequency or genepool level. Depending on the degree of such genetic differences populations may be identified as races, sub-species or new species. This stock concept from the point of view of fish population genetics was highlighted by Altukhov (1981).

The present most popular method adopted for identification of genetic stocks of commercially important fishery resources is gel-electrophoretic techniques developed by Smithies (1955) for detection of human serum protein genotypes and enzyme genotype detection method developed by Hunter and Markert (1957). These biochemical genetic techniques are widely applied in obtaining required basic genotype data in the form of protein/enzyme phenotypes and their variations. Many international maritime states have been applying these biochemical genetic techniques for differenciation and identification of natural unit stocks, with a view to scientific management and conservation of their commercially important fish resources. India is far behind in this specialized field of population genetics that forms a scientific basis of fisheries research and management. However, an awareness on the unique role of genetics in fisheries research management and conservation of fish resources of India was recently felt and highlighted by a few (Bye and Ponniah, 1983; Jhingran, 1984; Das and Jhingran, 1989).

The most important candidate of marine fish species of India to be investigated for identification of biochemical genetic stocks is the Oil Sardine, Sardinella longiceps. Because, in exploiting this commercially very important fish resource of India, many enigmatic problems are faced by the biologists and fishery management people. One of the most important problems facing the oil sardine fishery of India is the undesirable sudden fluctuations in its total landings throughout the past few decades (Antony Raja, 1973). It is exploited from both west and east coast of India, as if, a single stock fishery. The studies on its different aspects of fishery and biology revealed some form of population heterogeneity among its morphometric characteristics. Therefore, Devanesan and Chidambaram (1943), Prabhu and Dhulkhed (1972) and Antony Raja (1973) suspected the existence of races or sub-populations of oil sardine along the west coast of India. Naturally, these problems in oil sardine fishery of India may be the consequences of many unknown correlated factors, including genetic stock differences of regional populations supporting the fishery. Naturally, the primary question is: what is the population genetic composition of oil sardine fishery of India? Is it composed of genetically homogeneous or heterogeneous populations?

The major objective of the present investigation was to study the biochemical genetic nature of different populations of S. longiceps along the selected regions of west and east coast of India. The important and original findings made on the biochemical genetic characteristics of three populations from the west coast and two populations from the east coast of India have been presented in the thesis. A total of 25 enzyme loci belong-

ing to nine enzyme systems were examined to study the genetic nature of individual populations. The results are presented, described and discussed enzyme and loci wise to get an insight into population genetic structure of oil sardine Sardinella longiceps studied in the present investigation.

2. REVIEW OF LITERATURE

Accurate identification of species and its subunits or stocks is a prerequisite to the modern scientific management of fisheries resources. There is a large volume of literature published on the concept of STOCK in the field of fisheries research and management and various methods/techniques used for its identification.

In the early history of fisheries research, morphologically and meristically differentiated races were considered as practical units of fisheries management (Heinke, 1898; Schmidt, 1917). Later, the concept of stock and its different forms were introduced. The term fish stock has been defined and used in many contexts by different investigators, ranging from a production or management unit (Marr, 1957; Larkin, 1972) to that emphasises genetic discretness (Moller, 1971; Ihssen, 1977). Murray (1961) has explained variety of usage and different meanings of the word "stock". Ricker (1972) has explained that fish stocks probably have genetic individuality. The term sympatric stocks has been used by Ihssen et al. (1981a) to denote stocks that are not isolated by physical barriers. Booke (1981) has also reviewed the stock concept along with different definitions as applied in fishery science and also has presented a working definition of the "stock". His general definition of stock is "a species-group or population of fish that maintains and sustains itself over a time in a definable area". In this respect, the most popular unit stock concept applied in fisheries research and management is based on the Mendelian populations. It is defined as a "reproductive community of sexual and cross fertilized individuals among whom matings regularly occur

and who, consequently, have a common gene pool" (Dobszhansky, 1967; Altukhov, 1981). Naturally, gene controlled variations of proteins and enzymes have become the best source of informations on a common gene pool. A comparison of allelic frequencies in populations thus become the most common practice for identification of genetically distinct stocks (Allendorf and Phelps, 1981; Altukhov, 1981).

The identification of distinct genetic stocks, if any, is basic to the conservation and rational exploitation of fisheries resources (Leberg, 1990). Smith et al. (1990) argues that the biological stock concept developed with the studies on cod (Gadus morhua), herring (Clupea harengus) and plaice (Pleuronectes platessa). Critical importance of stock concept to the formulation of any comprehensive long term fisheries management has been emphasised by many investigators (Utter and Hodgins, 1972; Allendorf and Utter, 1979; Altukhov, 1981; Ihssen et al., 1981a; Larkin, 1981; Mac Lean and Evans, 1981; Phillipp et al., 1981; Allendorf et al., 1987; Kapuscinski and Philipp, 1988; Gulland, 1989; Lavery and Shaklee, 1989; Utter et al., 1989; Smith, 1990; Waples et al., 1990; Utter and Ryman, 1993).

Ihssen et al. (1981a) have reviewed some of the materials and methods that have been traditionally used to identify and delineate different stocks. They have discussed a wide variety of population parameters such as abundance, age composition, recruitment, mark recapture-procedures (tagging) etc. and various characters ranging from morphometric, meristic and calcareous to biochemical and cytogenetic characters which have been used to identify

fish stocks. Traditionally, studies on anatomical characters such as morphometrics and meristics have been conducted to identify different fish stocks. But phenotypic variations of these characters have not been directly correlated to particular differences in the genome and hence their application in stock identification is complicated (Clayton, 1981). Moreover, the effects of physiological and epigenetic constraints on morphology in response to certain environmental parameters such as temperature and oxygen are poorly understood (Bock, 1980; Todd et al., 1981). However, anatomical characters also have indicated stock differences which were agreeing to the data from alternative methods (Sharp et al., 1978; Casselman et al., 1981; Ihssen et al., 1981a).

Stock identification based on fish chromosome morphology has been done since 1945 (Booke, 1968) as reported by Ihssen et al. (1981a). Recently many modern practices have been evolved for isolation and resolution of fish chromosomes which are invariably of small size and great number. This enabled many researchers to adopt chromosome characters for stock delineation. However, Ihssen et al. (1981a) and Bye and Ponniah (1983) have indicated about the limitations in accepting intraspecific chromosomal variation as an indication of stock difference. Moreover, Boothroyd (1959) and Rees (1967) have reported that most of the intraspecific chromosomal differences are due to errors in methodologies or techniques rather than true genetic differences. Above all, live specimens are to be used for the extraction of chromosomes which may be difficult especially in the case of truly marine species like S. longiceps.

Realising the role of environmental parameters on the phenotypic expression in fishes, modern investigators started applying biochemical genetic techniques for identification of gene controlled phenotypes such as proteins and enzymes in different tissues of fishes. Biochemical techniques designed to compare species on the basis of protein differences dates back to 1904 when Nuttal used immunological methods to compare the serum of humans with that of other primates. Later on the classical paper electrophoresis work of Pauling et al. (1949) showed phenotypically different haemoglobins in human blood. Then the introduction of starch gel electrophoresis by Smithies (1955) demonstrated human serum protein variations. The innovation of starch gel zymogram methods by Hunter and Markert (1957) revealed a world of enzyme polymorphism in organisms ranging from Drosophila to humans. Meanwhile, Raymond and Weintraub (1959) introduced polyacrylamide as an effective electrophoretic medium. Its high resolving power due to adjustable pore size made it a better gel medium than starch gel. Then, Davis (1964) provided simple polyacrylamide disc gel electrophoretic unit as a popular method for protein/enzyme separation. Using both starch and polyacrylamide gel media, researchers in biology widely published the natural phenomenon of genetic variations in different proteins and enzymes. Shaw (1965) reviewed electrophoretic variation of enzymes in vertebrates and invertebrates. Harris (1966) detected polymorphic forms of many enzymes in man. Lewontin and Hubby (1966) and Hubby and Lewontin (1966) discovered high genetic variability within the species, Drosophila pseudoobscura. Meanwhile, attempts were made to apply blood groups and serum protein characteristics in fish

population genetic studies (Sick, 1961; Marr and Sprague, 1963; Cushing, 1964; de Ligny, 1969; Tsuyuki et al., 1969). Studies on serological and biochemical aspects involving eyelens protein, muscle protein and enzymatic proteins have been reviewed by de Ligny (1969).

The biochemical and serological methods have been discussed with special reference to identification of fish stocks during the ICES special meeting held at Dublin (de Ligny, 1971). It is evident from different reports that general proteins and some enzymatic proteins were useful for stock identification programmes. Among general proteins, muscle proteins and eyelens proteins were widely used for stock identification studies by most of the investigators (Tsuyuki et al., 1965a,b, 1968; Tsuyuki and Roberts, 1966; Eckroat and Wright, 1969; Uthe and Ryder, 1970; Peterson and Shehadeh, 1971; Menezes, 1976a,b; Rao and Dhulkhed, 1976; Jamieson and Turner, 1980; Smith et al., 1980; Winans, 1980; Andersson et al., 1983; Fujio et al., 1983; Basiao and Taniguchi, 1984; Sbordoni et al., 1986; Smith, 1986; Mahobia, 1987; Philip Samuel, 1987; Present, 1987; Feveldon and Haug, 1988; Salini and Shaklee, 1988; Menezes, 1990; Vijayakumar, 1992).

The electrophoretic techniques and staining procedures for detection of different enzyme systems published by Shaw and Koehn (1968), Brewer (1970), Shaw and Prasad (1970) enabled different researchers to discover and publish large volume of informations on electrophoretic characteristics of different enzyme systems. Publication of books on electrophoresis with different methods and techniques to detect atleast 40 enzyme systems (Smith,

1968, Brewer, 1970), laboratory manual or practical guide specifically designed to detect electrophoretic variations of these enzymes in prawns (Siciliano and Shaw, 1976; Redfield and Salini, 1980), fishes and shell fishes (Benson and Smith, 1989) and method of interpretation of electrophoretic phenotypes as genetic variants and for purpose of stock identification of fish (Utter et al., 1987) greatly helped in applying biochemical genetic techniques in the fisheries research and management. The very basis of identification of distinct genetic stocks is the expected evolutionary processes occuring within the species. The degree of evolutionary diversification between identified discrete stocks may also be expressed in terms of genetic identity and genetic distance measured according to the method of Nei (1972). Its application in biochemical genetics of populations was explained using models (Utter, 1987; Ayala and Kiger, 1980).

The major reason for world wide application of biochemical genetic techniques involving gel electrophoresis is the implication of genetic stock concept in fisheries management (Moller, 1968, 1970; de Ligny, 1969, 1972; Utter et al., 1974; Allendorf and Utter, 1979). Since most of the biochemical processes involve catalytic participation of large number of enzyme systems, they have been investigated as potential source of genetic variation studies. Thus major portion of literature available on modern fish stock identification studies are based on enzyme allelic frequencies. Among different group of enzyme systems, dehydrogenases are studied in detail for stock identification programmes, especially in different species of salmon and trout by many of the investigators (Hodgins et al., 1969; Northcote et al., 1970; Williscroft

and Tsuyuki, 1970; Wright and Atherton, 1970; Utter and Hodgins, 1972; Aspinwall, 1973; Utter et al., 1973; Allendorf et al., 1976; Bailey et al., 1976; Busak et al., 1980; Grant et al., 1980; Wishard et al., 1980; Dehring et al., 1981; Guyomard, 1981; Stoneking et al., 1981; Utter, 1981; Carl and Healy, 1984; Guyomard et al., 1984; Kreig and Guyomard, 1985; Milner et al., 1985; Thompson, 1985; Withler, 1985; Koljonen, 1986; Okazaki, 1986; Campton and Utter, 1987; Gharrett et al., 1987; Marnell et al., 1987; Quinn et al., 1987; Skaala and Jorstad, 1987; Wehrahn and Powell, 1987; Berg and Gall, 1988; Verspoor, 1988; Utter et al., 1989; Crozier and Moffett, 1990; Currens et al., 1990; Macaranas and Fujio, 1990; Hershberger, 1992; Utter et al., 1992).

Using dehydrogenases, many other fishes have also been thoroughly investigated for stock identification such as New Zealand snapper (Smith et al., 1978), walleye pollock (Grant and Utter,1980), Coregonus clupeaformis (Casselman et al., 1981), jackmackerel (Richardson, 1982a), jackass (Richardson, 1982b), Atlantic herring (Grant, 1984), damsel fish (Shaklee, 1984), paci fic herring (Grant and Utter, 1984), Atlantic cod (Mork et al., 1985).

In addition to dehydrogenase enzymes, esterase enzyme system has also been investigated in large number of fishes like cat fish (Koehn and Rasmussen, 1967; Koehn, 1970), Pacific hake (Utter et al., 1970), American eel (Williams et al., 1973), European hake (Mangaly, 1974; Mangaly and Jamieson, 1978), sun fish (Avise and Smith, 1974), Zoarces (Christiansen and Frydenberg, 1974), Cichlids (Kornfield and Koehn, 1975; Mahobia, 1987),

Menidia (Johnson, 1975), guppy (Shami and Beardmore, 1978), New Zealand snapper (Smith et al., 1978; Smith, 1979), Atlantic mackerel (Smith and Jamieson, 1980; Smith et al., 1981a), milk fish (Winans, 1980), walleye pollock (Grant and Utter, 1980), sprat (Smith and Robertson, 1981; Ryman and Stahl, 1981), New Zealand hoki (Smith et al., 1981b), Catostomus (Buth and Crabtree, 1982), paddle fish (Carlson et al., 1982), tilapia (Cruz et al., 1982; Mc Andrew and Majumder, 1983; Basiao and Taniguchi, 1984); Australian barramundi (Shaklee and Salini, 1983; Salini and Shaklee, 1988), Orizias (Sakaizumi et al., 1983), Salvelinus (Andersson et al., 1983), Atlantic herring (Grant, 1984), Pacific herring (Grant and Utter 1984), perch (Gyllesten et al., 1985), Northern pike (Seeb et al., 1987), Pacific cod (Grant et al., 1987), shark (Lavery and Shaklee, 1989), tuna (Richardson and Habib, 1987), grey mullet (Vijayakumar, 1992) and crustaceans (Johnson et al., 1974; Lester, 1979; Kannupandi, 1980; Mulley and Latter, 1980; De Matthaeis et al., 1983; Philip Samuel, 1987).

Studies on biochemical genetics of Indian fishes are only a few and are of preliminary nature. These were mainly attempts to find out inter-species or species specific protein differences. Some of the examples are, flat fish (Kasinathan et al., 1972), marine fishes (Manohar and Velankar, 1973), goboids (Natarajan et al., 1975), Bombay duck (Kurian, 1977), oil sardine (Rao and Dhulkhed, 1976), mackerel (Dhulked and Rao, 1976; Menezes, 1986; Menezes et al., 1990), M. cephalus (Bhosle, 1977), mullets (Rao, 1981), Channa stewartii and Danio dangila (Bhattacharya and Alfred, 1982), Etroplus suratensis Liza macrolepis and Mystus gulio (Kamalakara Rao et al., 1985), grass carp

(Padhi and Khuda-bukhsh, 1989), carangids (Menezes, 1990). Similar investigations have also been carried out in shrimps and oysters (Sriraman and Reddy, 1977; Kulkarni et al., 1980; Thomas, 1981; Puthran Prathibha, 1984; Ponniah, 1988).

However, there are also a few Indian reports on the biochemical genetics of fishes and shrimps with due importance to stock identification studies, namely, mullets (Reddy, 1977; Rao, 1981), cichlids (Mahobia, 1987), penaeid prawns (Philip Samuel, 1987) and Mugil cephalus (Vijayakumar, 1992). The application of genetics in aquaculture was also emphasised in the C.M.F.R.I. Special publication (Bye and Ponniah, 1983). An awareness on the importance of fish genetic resources, its conservation and management in India has been published by Jhingran (1984), Das and Jhingran (1989) and Das et al. (1989).

The above review of the literature gives sufficient justification for the choice of the present thesis problem, its objectives, its methods of investigation/interpretation of data and the conclusions drawn from the results of the investigation.

3. MATERIALS AND METHODS

3.1 MATERIALS

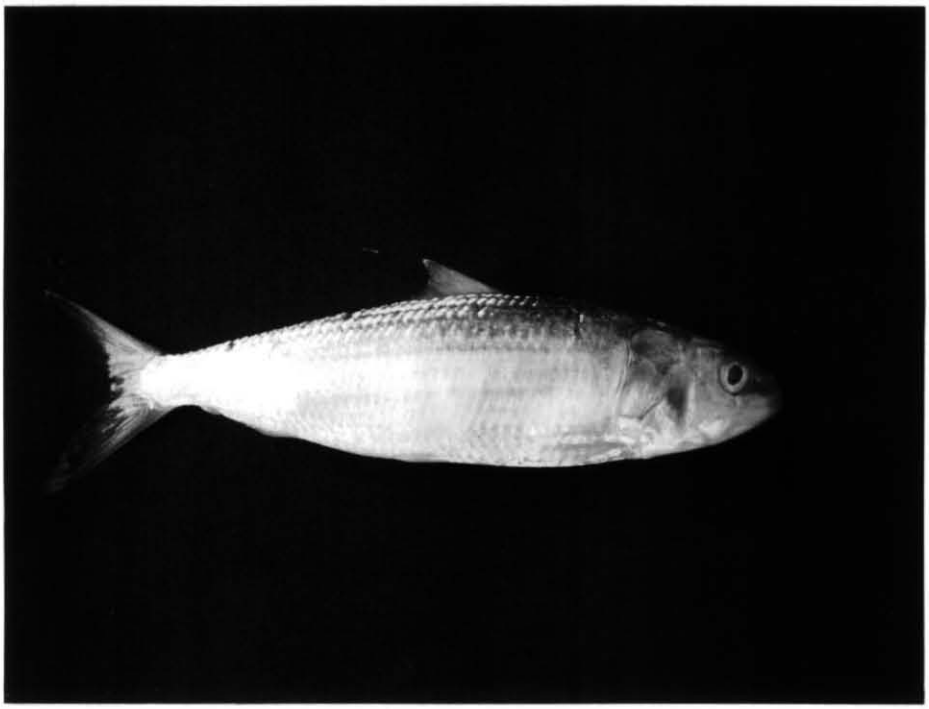
3.1.1 THE EXPERIMENTAL ANIMAL

The Indian oil sardine, Sardinella longiceps, the experimental animal in the present study was collected from five different geographical areas, along the west and east coast. West coast samples were drawn from Cochin, Calicut and Mangalore whereas east coast samples were collected from Mandapam and Madras (FIG.1; PLT.1). The oil sardine samples were collected either on-board from purse-seiners or from landing centres. Samples from Cochin were collected mostly from purse-seiners off Cochin. Mandapam population samples were collected using a shore seine net. The length and weight of specimens used for experimental analysis ranged between 15-20 cms and about 25-30 gms respectively. From each specimen, four tissues, namely heart, liver, muscle and eye lens were taken for studies. Heart and liver tissues were washed in ice-cold Double Distilled Water (D.D.W.) and dried with blotting paper in order to render them free from any blood stains.

3.1.2 SAMPLE TRANSPORTATION

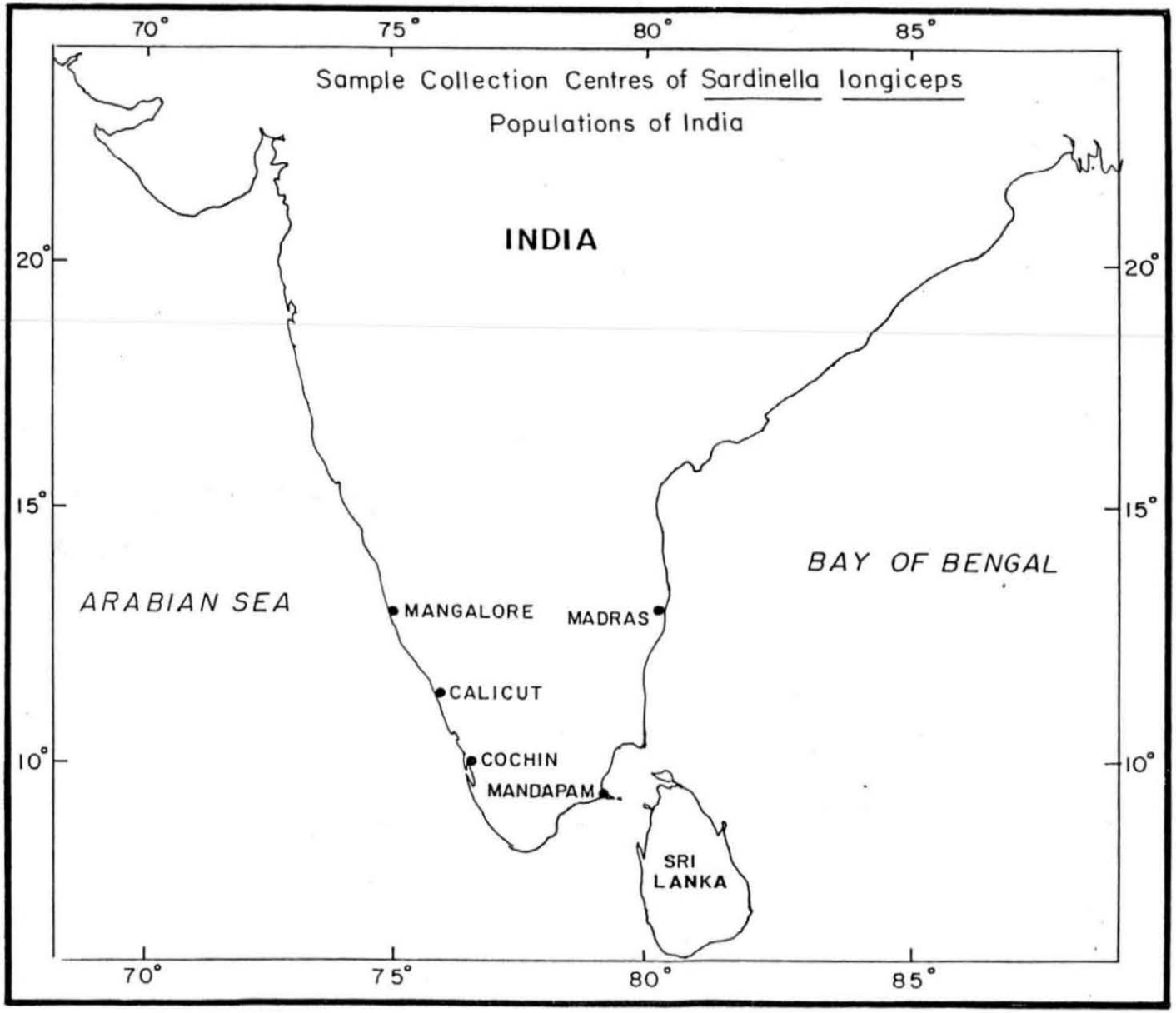
Fish sample (whole specimen) from Cochin and Calicut regions was transported to the laboratory in iced condition. Samples from Mangalore, Madras and Mandapam were brought to the laboratory in the form of tissues packed in the aluminium foil and kept in plastic vials. These vials were stored in an ice box containing ice. The ice box as it is, was kept in a deep freezer available in the nearby area with a layer of water above the

PLATE 1



Sardinella longiceps

FIG. 1


70°
75°
80°
85°
Sample Collection Centres of Sardinella longiceps
Populations of India
INDIA
20°
15°
10°
ARABIAN SEA
BAY OF BENGAL
MANGALORE
MADRAS
CALICUT
COCHIN
MANDAPAM
SRI
LANKA

ice until a block of ice was formed within the ice box. The ice box was immediately transported to Cochin laboratory where the samples were stored in the deep freezer until used for experimental analysis.

3.2 METHODS

3.2.1 PREPARATION OF TISSUE HOMOGENATE

Once the specimens or tissue samples have been obtained, an enzyme extract of each tissue was prepared from each specimen. The tissues kept in deep freezer were allowed to thaw for about 10-15 minutes, weighed and homogenized with D.D.W. at the rate of 100 mg/1 ml for all tissues except muscle where 80 mg/1 ml was used. Homogenization was done in Remi Mechanical Homogenizer. The homogenized tissues were centrifuged in Sorvall Refrigerated Super Speed Centrifuge for 20 minutes in 10,000 rpm at 5°C. The supernatants obtained were used for electrophoretic analysis.

3.2.2 POLYACRYLAMIDE GEL ELECTROPHORESIS

The principle of electrophoresis is that charged ions or group of ions will migrate towards one of the electrodes when placed in an electric field, according to their net charge and molecular size. The rate of migration varies for different ions or groups of ions and this difference is used to separate the components of a protein mixture. The positively charged Cations will move to the cathode whereas negatively charged Anions will move to anode. Since most of the proteins are negatively charged, the protein mixture is applied at cathode region, which then migrate towards the opposite positively charged anode region. The method of electrophoresis followed here was that of Dietz et al. (1972).

3.2.3 PROCEDURE FOR DISC GEL ELECTROPHORESIS

Reagents

The following reagents were prepared as follows and were stored in refrigerator at 4°C until used.

Acrylamide Stock Solution (40%):

40 gm of acrylamide was dissolved in D.D.W. and made upto 100 ml and filtered. This filtered solution was stored in an amber coloured bottle.

Bis Acrylamide (2.1%):

2.1 gm of N, N^1 - methylene - bisacrylamide was dissolved in D.D.W and made upto 100 ml and filtered.

Ammonium Persulphate (0.14%):

140 mg of ammonium persulphate was dissolved in D.D.W. and made up to 100 ml.

Marker Dye:

100 mg of bromophenol blue was dissolved in 100 ml of D.D.W. and filtered. Sucrose solution was prepared by dissolving 40 gm. of sucrose in 100 ml of D.D.W. 1 part of filtered bromophenol blue was mixed with 9 parts of sucrose solution. This solution was used as the tracer dye for the experiment.

3.2.4 BUFFER SYSTEMS

Three different buffer systems were used for the electrophoretic separation and resolution of enzymes tested here. The details are given below.

I. TRIS-CITRATE (Shaw and Prasad, 1970)

Electrode buffer

Tris	=	16.35 gm
Citric acid	=	9.04 gm

Both the components were dissolved in one litre of D.D.W and the pH was adjusted to 7.0. Modification of pH was done from Shaw and Prasad (1970)(pH = 7.1).

Gel buffer

6.67 ml of electrode buffer was diluted to one litre and the pH was adjusted to 7.0

Modification of pH was done from Shaw and Prasad (1970)(pH = 7.1).

II. TRIS-VERSENE BORATE (Shaw and Prasad, 1970)

Electrode buffer

Tris	=	60.60 gm
Boric acid	=	40.00 gm
$Na_2EDTA.2H_2O$	=	6.00 gm

The components were dissolved in one litre of D.D.W and the pH was adjusted to 8.0.

Gel buffer

Tris	=	6.06 gm
Boric acid	=	6.00 gm
$Na_2EDTA.2H_2O$	=	0.60 gm

The components were dissolved in one litre of D.D.W. and the pH was adjusted to 8.0.

III. TRIS-CITRATE/LITHIUM HYDROXIDE-BORIC ACID (Ridgway et al., 1970)

Electrode buffer

Lithium Hydroxide	=	2.5 gm
Boric acid	=	18.5 gm

The components were dissolved in one litre of D.D.W. and the pH was adjusted to 8.21.

Modification of pH was done from Ridgway et al. (1970) (pH = 8.0).

Gel buffer

Tris	=	3.6 gm
Citric acid	=	1.0 gm

The components were dissolved in one litre of D.D.W. and the pH was adjusted to 8.31.

Modification of pH was done from Ridgway et al. (1970) (pH = 8.0).

To all the gel buffers 0.23 ml of TEMED for every 100 ml of gel buffer was added for polymerisation before casting the gel.

STAINING BUFFER (Tris-HCl, pH=7.1)

60.75 gm of Tris was dissolved in one litre of D.D.W. and the pH was adjusted to 7.1 using conc. HCl.

3.2.5 GEL CASTING

The stock solutions like acrylamide, bisacrylamide, gel buffers and ammonium persulphate were brought to room temperature before casting

the gel. Throughout the experiment 7.0% gel concentration was used. To obtain 7.0% gel, 3.5 ml of acrylamide solution, 2.0 ml of bis acrylamide solution, 4.5 ml of D.D.W., 5.0 ml of gel buffer and 5.0 ml of ammonium persulphate solution were thoroughly mixed. The gel tubes fixed in the gel tube stand were carefully filled with the above gel mixture with the help of a glass syringe along the sides of the gel tube to avoid bubble formation. Two to three drops of D.D.W. was layered over the gel top to avoid miniscus formation. The gel gets polymerized within 20 to 30 minutes at room temperature after which the water layer was decanted.

3.2.6 ELECTROPHORESIS

When the gel is ready, 40 µl of the marker dye was added on the top of the gel. The clear supernatent portion of the homogenate was then added and throughly mixed with the marker dye. The sample volume differed for different enzymes (TBL:1). The remaining part of the gel tube was filled with the electrode buffer. The marker dye helps in knowing the completion of the electrophoretic run. Besides, the sucrose solution present in the dye helps to avoid mixing of the sample solution with the tank buffer. It also ensures uniform flow of current and faster migration of the sample into the gel. The gel tubes were then removed and inserted into the rubber grommets of the upper buffer tank. Both the buffer tanks were filled with the electrode buffer. The lower buffer tank was attached with anode (+ve) lead of the power pack. Then the upper tank was fixed on to the lower tank of the apparatus and attached with cathode (-ve) lead of the power pack.

The power pack was switched on for 10 minutes before starting the electrophoresis. Initially the current was regulated to have an uniform flow of 12 milliampere (mA) for the first 10 minutes and then adjusted to 36 mA till the completion of experiment. The apparatus was disconnected when the dye reaches the mark made on the lower end of the gel tube. The gels were then removed from the gel tubes by forcing a jet of water between the gel and the inner wall of the gel tube using a syringe without damaging the gels.

3.2.7 STAINING PROCEDURE

Since most proteins are colourless, the separated enzyme proteins were made visible after the incubation of gels in a specific enzyme staining solution. The staining solutions contained the substrate for the specific enzyme, cofactor NAD, electron indicator MTT and electron acceptor PMS in a staining buffer (0.5 M Tris-HCl, pH = 7.1). The following staining solutions were used for detection of specific enzymes.

ALCOHOL DEHYDROGENASE (Guyomard, 1981)

Modified

Tris-HCl (0.5 M, pH=7.1)	=	30 ml
M T T	=	7 mg
N A D	=	8.5 mg
Propan-2- Ol	=	2 ml
P M S	=	1 mg

ALDEHYDE OXIDASE (Redfield and Salini, 1980)

Tris-HCl (0.5 M, pH=7.1)	=	15	ml
M T T	=	3	mg
N A D	=	6	mg
Benzaldehyde	=	0.30	ml
D.D.W.	=	15	ml
P M S	=	1	mg

ESTERASE (Shaw and Prasad, 1970)

Substrate solution : 1% α-β- Naphthyl acetate

α - Naphthyl acetate	=	1	gm
β - Naphthyl acetate	=	1	gm
Acetone	=	50	ml
D.D.W.	=	50	ml

Stain

Tris-HCl (0.5 M, pH=7.1)	=	3	ml
Fast Blue RR salt	=	30	mg
1% α-β- Naphthyl acetate	=	0.90	ml
D.D.W.	=	26.1	ml

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (Siciliano and Shaw, 1976)

Modified

Tris-HCl (0.5 M, pH=7.1)	=	30	ml
M T T	=	15	mg
N A D P	=	21	mg
$MgCl_2$	=	30	mg
Glucose-6-Phosphate	=	60	mg

GLUTAMATE DEHYDROGENASE (Shaw and Prasad, 1970)

Modified

Tris-HCl (0.5 M, pH=7.1)	=	30	ml
M T T	=	10	mg
N A D	=	20	mg
L-Glutamic acid	=	1.5	gm

ISOCITRATE DEHYDROGENASE (Benson and Smith, 1989)

Modified

Tris-HCl (0.5 M, pH=7.1)	=	30	ml
M T T	=	6	mg
N A D P	=	10	mg
$MnCl_2$	=	15	mg
DL-Isocitric acid	=	30	mg
P M S	=	1	mg

LACTATE DEHYDROGENASE (Guyomard, 1981)

Tris-HCl (0.5 M, pH=7.1)	=	30	ml
M T T	=	2	mg
N A D	=	4	mg
Lithium Lactate	=	60	mg
P M S	=	1	mg

MALATE DEHYDROGENASE (Siciliano and Shaw, 1976)

Modified

Tris-HCl (0.5 M, pH=7.1)	=	30	ml
M T T	=	12	mg

N A D	=	15	mg
Na_2CO_3	=	7	mg
Malic acid	=	15	mg
P M S	=	1	mg

XANTHINE DEHYDROGENASE (Shaw and Prasad, 1970)

Substrate solution : 1 M Hypoxanthine

Hypoxanthine	=	6.8	gm
1 M KOH	=	10	ml
D.D.W.	=	40	ml

Stain

Tris-HCl (0.5 M, pH=7.1)	=	6	ml
M T T	=	9	mg
N A D	=	18	mg
1 M Hypoxanthine	=	1	ml
D.D.W.	=	23	ml
P M S	=	1	mg

3.2.8 ZYMOGRAM AND PHOTOGRAPHY

The zymogram pattern for the enzymes was prepared by drawing the band patterns according to their position in the gels. The gels were kept in clean test tubes and photographed.

3.2.9 STANDARDIZATION OF EXPERIMENTAL CONDITIONS

Three different buffer systems were used to obtain good resolution and separation of the bands. To find out the best tissue source for each

enzyme, four different tissues such as heart, liver, muscle and eye lens were tested and compared. The optimum quantity of the sample to be extracted (mg/ml) and the homogenates to be loaded (µl) in a gel were also standardized. The standardized experimental conditions adopted for the screening tests for nine different enzyme systems are shown in Table 1.

3.2.10 PROCEDURE FOR ANALYSIS OF DATA

1. Interpretation of zymogram patterns

The zymogram patterns (phenotypes) for a particular enzyme system at a particular gel area between individuals in a sample were compared by adopting the following criteria. The phenotypic variants at an assumed locus were designated as slow moving **S** band (slow homozygote), fast moving **F** band (fast homozygote) and their combination as **SF** band (heterozygote) depending on the distance migrated by the particular band in that particular compared gel area. The band nearer to the origin of the gel which has moved less distance is considered as **S** band. The one which has moved faster than **S** band was designated as **F** band. As a standard practise, these gene controlled protein phenotypes were considered as genotypes produced by co-dominant alleles at a particular genetic locus. Depending on the enzyme structure, the observed heterozygote patterns were again generally scored as monomeric if two banded, dimeric if three banded and tetrameric if five banded.

2. Allele frequencies (TBL:2)

The first step taken in analysing the set of electrophoretic data was to calculate the individual genotypes observed for each locus of a particular

enzyme in each population sample. Allele frequencies were determined from the genotypic frequencies as follows:

Frequency of **S** allele is the summation of the frequency of **SS** genotype and half the frequency of **SF** genotype. Similarly the frequency of **F** allele is the sum of the frequency of **FF** genotype and half the frequency of **SF** genotype.

Allelic frequency can also be calculated using the formula:

$$\frac{\mathbf{Ho \times 2 + He}}{\mathbf{2\ N}}$$

Where Ho = observed number of a particular homozygote (either **SS** or **FF**)

He = observed number of a particular heterozygote (**SF**)

N = total number of individuals tested

3. Expected genotype frequencies (TBL:3)

The Hardy - Weinberg law provides a base for calculating the expected genotype proportions in different populations. The following binomial expression was applied to find out the expected genotypic frequencies.

$$\mathbf{(a+b)^2 = a^2 + 2\ ab + b^2}$$

The significance of deviation between the observed and expected genotype frequencies was calculated for determining the chi-square values using the formula

$$\frac{\mathbf{(Observed - Expected)^2}}{\mathbf{Expected}}$$

The degree of freedom for a diallelic three phenotype system was calculated using the formula

3 phenotypes	-	1	=	2
2 Alleles	-	1	=	1
The degrees of freedom	=	2-1	=	1

4. Heterozygosity (TBL:4)

The average number of heterozygotes present in all the loci of the population tested is heterozygosity. Initially, heterozygosity was calculated for each locus of a particular enzyme system in all the populations from which the average for each enzyme and there by average heterozygosity for the species. Both polymorphic and non-polymorphic loci tested were considered for calculation.

5. Average number of alleles (TBL:8)

$$\textbf{The average number of alleles per locus} = \frac{\textbf{Number of different alleles}}{\textbf{Number of loci tested}}$$

From this average number of alleles for different loci in each population was calculated. The average for the species was calculated by taking the average value for all the loci in all the populations tested.

6. Polymorphic loci (TBL:7)

Proportion of polymorphic loci is the fraction of loci that are polymorphic of all the loci examined. A locus was considered polymorphic when the most common allele had a frequency of 0.95 or less. The average of polymorphic loci for each enzyme in each population followed by the average for all the enzymes of the different populations, from which the average for the species was calculated.

7. Genetic identity (I) and Genetic distance (D) (TBL:10)

The genetic distance is the measure of accumulated allele differences per locus which is based on the identity of genes between the populations.

Genetic distance is defined as

$$D = - \log_e I$$

Where **I** is the normalized identity of genes between two populations and was calculated using the following formula

$$I = \lesssim X_i \; Y_i \; / \sqrt{X_i^2 \;\; Y_i^2}$$

where values of X_i and Y_i are the frequencies of specific alleles in the populations **X** and **Y** respectively.

The average **I** value for all the loci considered was calculated, from which the average **D** value for that particular comparison was derived. Then the average **I** and **D** values for all the comparisons made was found. The average for the species was estimated from all the compared average values of **I** and **D.**

4. RESULTS

Results of the present investigation are presented in alphabetical order of nine individual enzymes studied. Each enzyme system is described giving emphasis to phenotype/genotype nature and its frequencies at each locus in each population sample. At the end of the Results section, details on the average polymorphic loci, average alleles per locus, average heterozygosity and average genetic identity (I) and distance (D) have been presented.

4.1 ENZYME SYSTEMS (ALLELE FREQUENCIES)

4.1.1 ALCOHOL DEHYDROGENASE (ADH)

Figure 2 and Plate 2 show zymogram patterns of alcohol dehydrogenase (ADH) in Sardinella longiceps tested from Cochin, Calicut, Mangalore, Mandapam and Madras. The zymogram showed two different zones of enzyme activity which were presumed to be under the control of two independent loci. They were designated as ADH-I and ADH-II according to their order of increasing mobility differences. ADH-I locus occured at the top gel position (0-4 mm). The enzyme expression between 10 mm and 16 mm was considered as ADH -II locus. ADH-I locus was monomorphic in Cochin, Mangalore and Madras populations where only a single invariant slow band (**S** band) was observed. But calicut and Mandapam populations showed polymorphism at ADH-I locus. The polymorphism at ADH-I locus produced slow type homozygote (**S** band) and two banded slow fast type (**SF** band) heterozygotes. However, fast type (**F** band) homozygotes were not observed in these two regions.

The enzyme patterns at ADH-II locus appeared almost identical to that of ADH-I. The ADH-II also showed polymorphism in Calicut and Mandapam populations and monomorphism in Cochin, Mangalore and Madras populations.

FIGURE 2 AND PLATE 2

Zymogram patterns of Alcohol Dehydrogenase

in S. longiceps populations from

A	-	Cochin	Locus		
B	-	Calicut	I	=	0-4 mm
C	-	Mandapam	II	=	10-16 mm
D	-	Mangalore/Madras			

FIG. 2

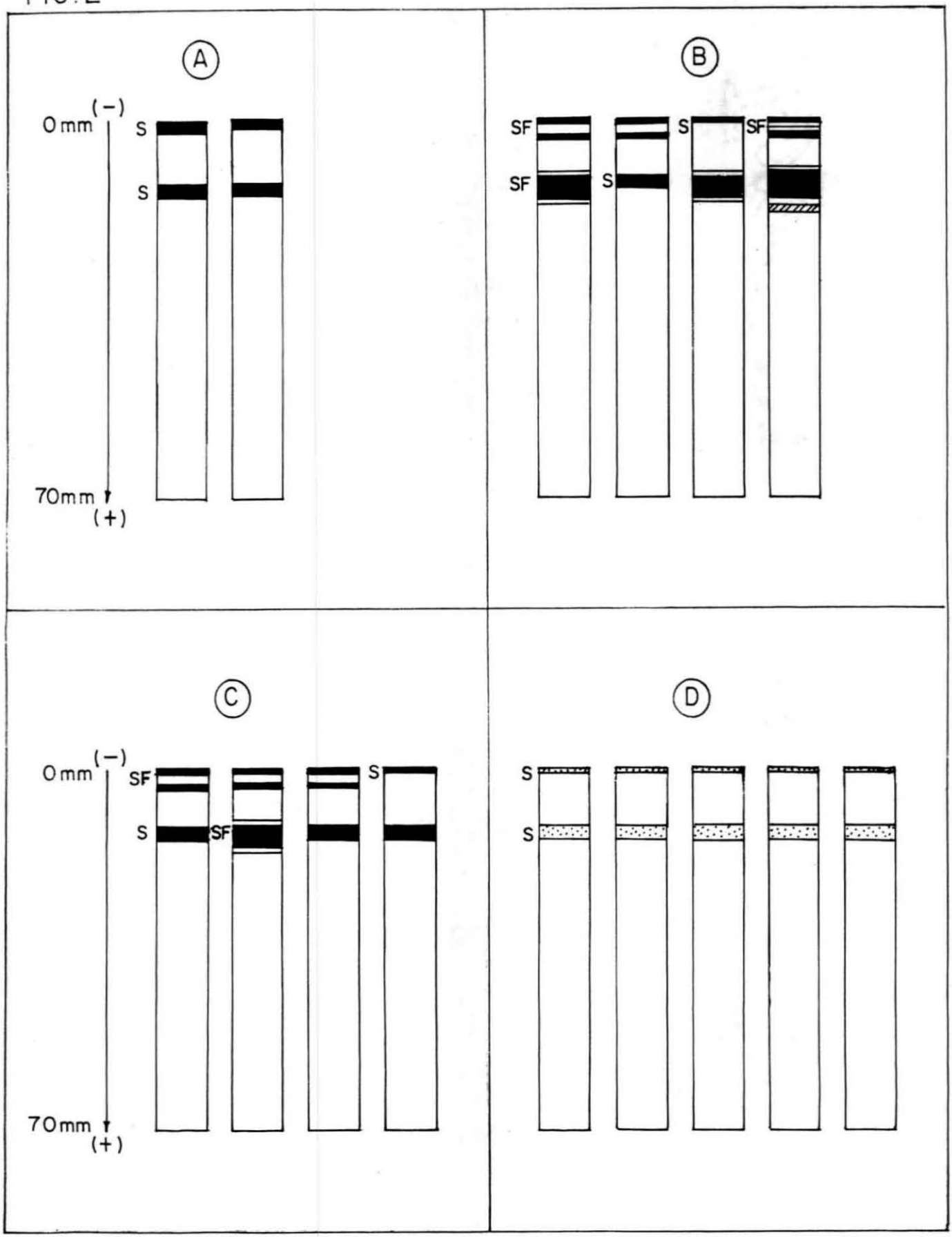
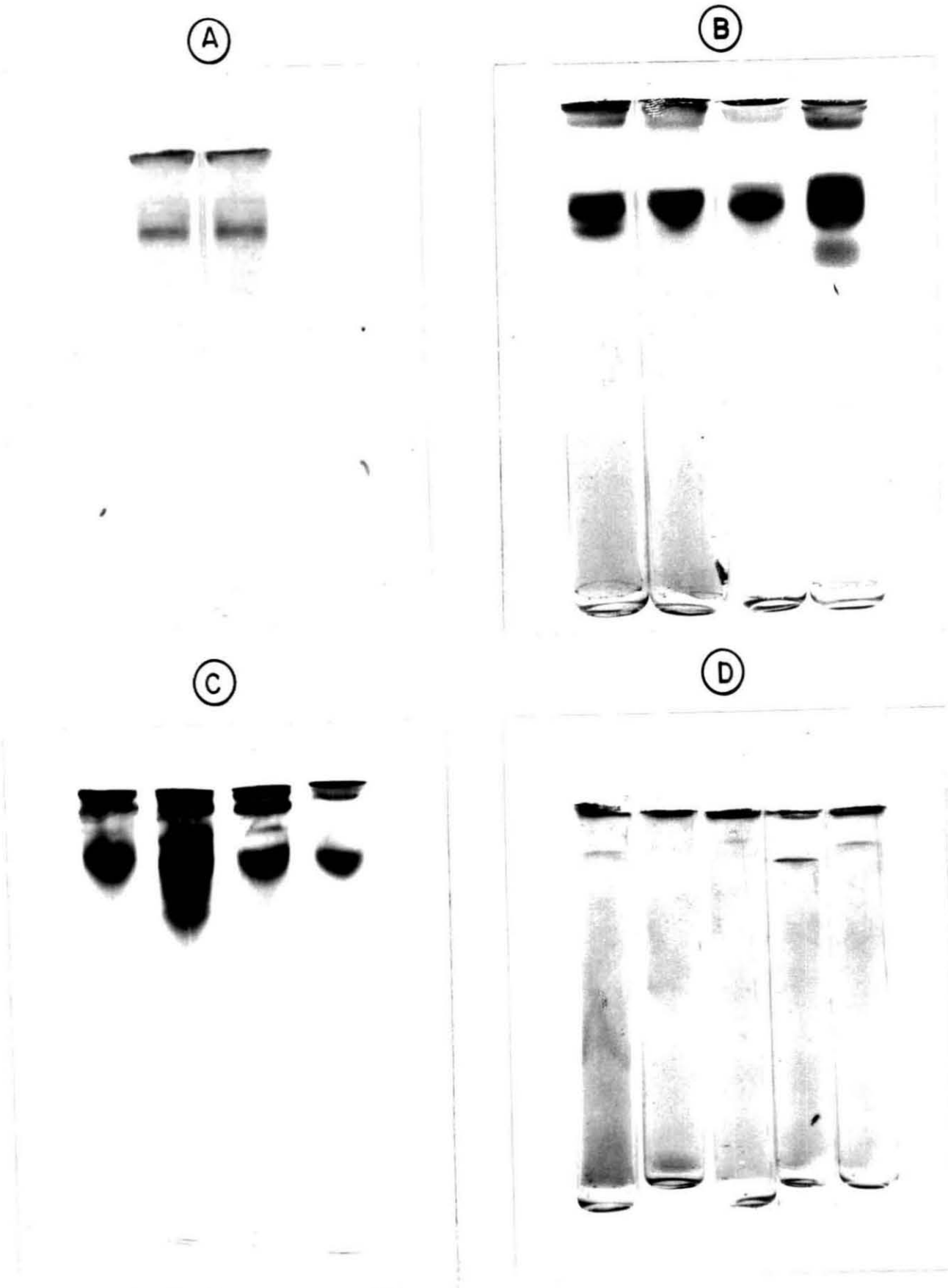


PLATE 2



In Calicut and Mandapam populations slow homozygous and slow/fast heterozygous phenotypes were observed whereas fast homozygotes were absent.

Both ADH-I and II loci show single band while monomorphic and triple band while polymorphic. At ADH-I locus the intermediate band was not so clear in some tubes. However, the intermediary band was very clear in fourth tube of the photograph (PLT. 2B). Though three bands were present in heterozygous condition at ADH-II locus in some specimens from Calicut and Mandapam, the bands above and below the major band were not sharp and clear (PLT.2B,C). In Mandapam population the only tube that showed three banded heterozygote condition was the second tube (PLT.2C). Also in general the ADH-II locus bands were not sharp and compact as that of Calicut. This may be due to the deformity occured either during casting or when taking the gel out for staining as regard to Madras population, the activity of the enzymes at both the loci was less and therefore the bands were not as thick as that of other regions. However, the zymogram of Madras population clearly shows the observed band position (FIG.2D).

The allelic frequencies of alcohol dehydrogenase first and second loci are shown in the Table 2. Populations from Cochin, Mangalore and Madras showed cent percent **S** allele at both ADH loci. Calicut and Mandapam populations showed both **S** and **F** alleles. However, the predominant allele in these populations was **S.**

A close comparison of the nature of allelic frequencies at both ADH loci in populations revealed an interesting phenomenon of stock differences. For example allelic frequencies of both loci between Cochin/Calicut, Calicut/

Mangalore and Mandapam/Madras are significantly different inspite of their closeness. Cochin/Mandapam differed significantly in their allelic frequencies. In short, though the allelic frequencies themselves are identical among Cochin, Mangalore and Madras populations and these frequencies are comparable between Calicut and Mandapam, occurence of heterogeneous populations having different allelic frequencies between similar populations appear to cause a barrier for mixing or inter breeding of homogeneous populations (TBL.2).

The values of observed and expected genotypic distributions in different populations at ADH-I and II loci are summarized in the Table 3. The expected values for ADH-II locus in Calicut and Mandapam populations are in agreement with the Hardy-Weinberg proportions.

4.1.2 ALDEHYDE OXIDASE (AO)

Figure 3, 4 and Plate 3, 4 show zymogram patterns of Aldehyde oxidase (AO) in oil sardine, Sardinella longiceps sampled from Cochin, Calicut, Mangalore, Mandapam and Madras. Zymogram pattern shows three different zones of enzyme activity which are referred to as three loci. They are AO-I, AO-II and AO-III. First locus at 0-2 mm position showed only one band in all the populations tested and therefore the locus was considered as non-polymorphic. The second locus AO-II between 6 mm and 9 mm showed polymorphism in all the five populations. All the specimens from Calicut and Mangalore however showed only double banded heterozygotes whereas Cochin, Mandapam and Madras populations showed homozygote and heterozygote band patterns. In Cochin population, fast type (**F** band) homozygotes and slow/fast

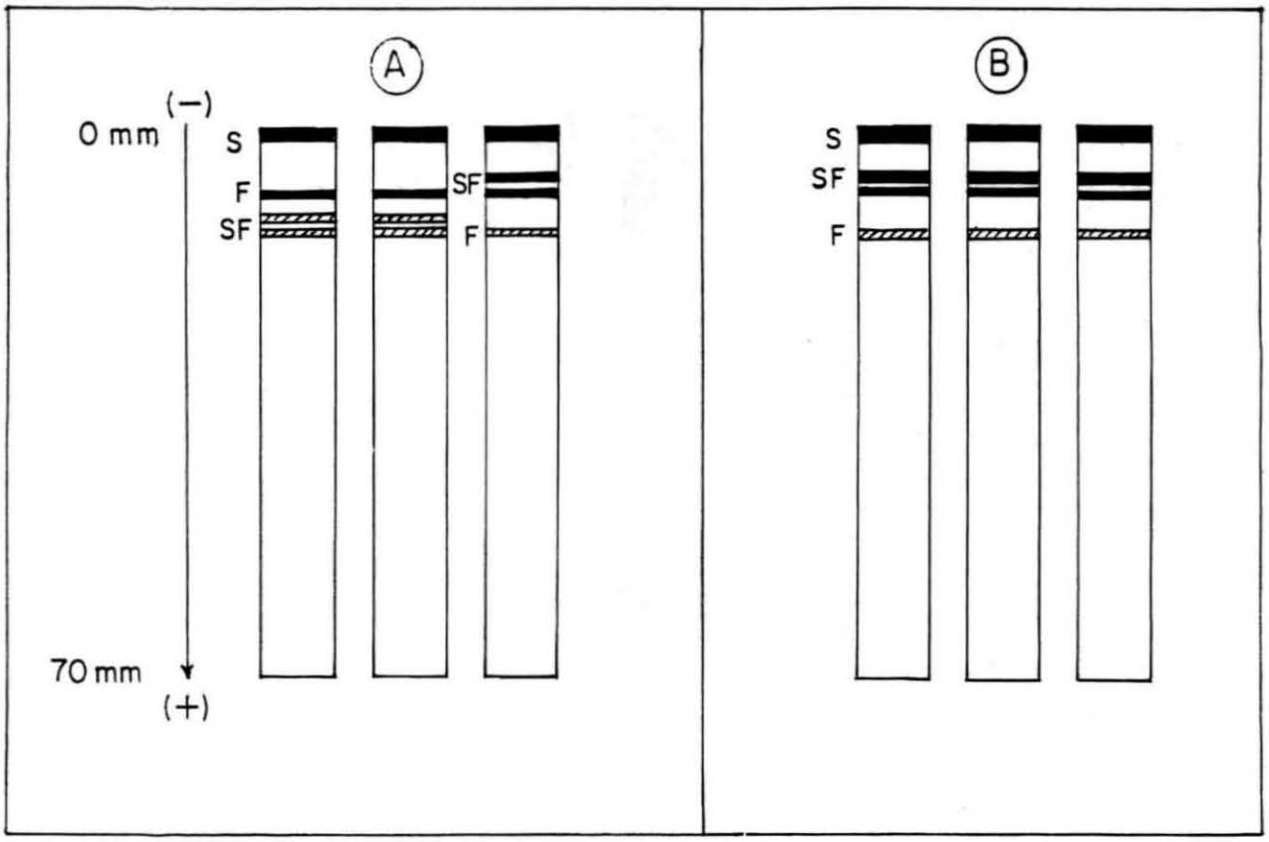
FIGURE 3 AND PLATE 3

Zymogram patterns of Aldehyde Oxidase in

S. longiceps populations from

			Locus			
A	-	Cochin	I	=	0-2	mm
B	-	Calicut	II	=	6-9	mm
C	-	Mangalore	III	=	12-14	mm

FIG. 3



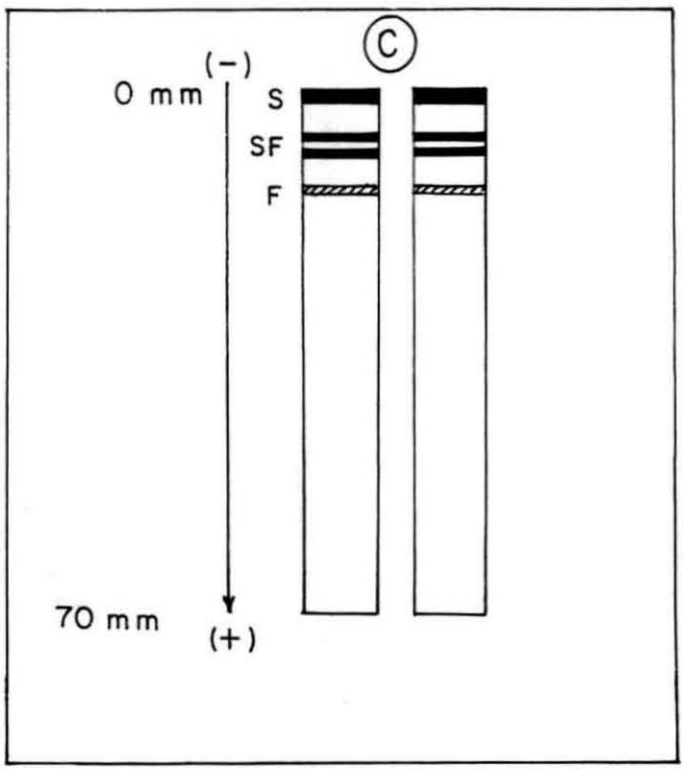


PLATE 3

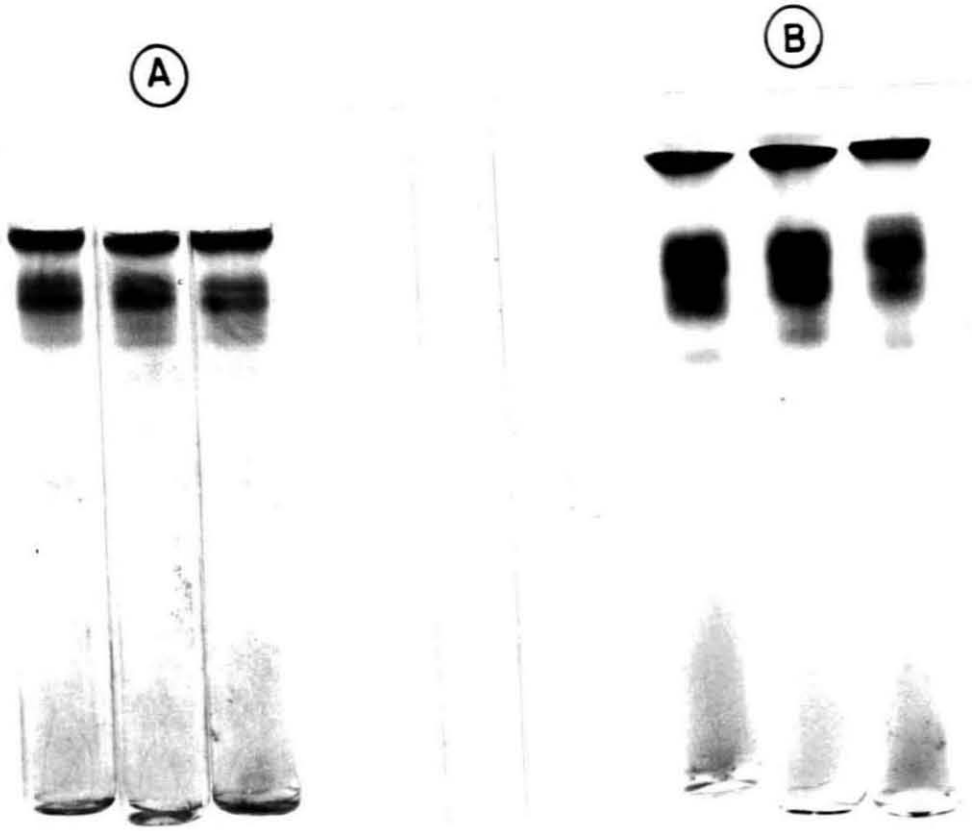


FIGURE 4 AND PLATE 4

Zymogram patterns of Aldehyde Oxidase

in S. longiceps populations from

			Locus			
D	-	Mandapam	I	=	0-2	mm
E	-	Madras	II	=	6-9	mm
			III	=	12-14	mm

FIG. 4

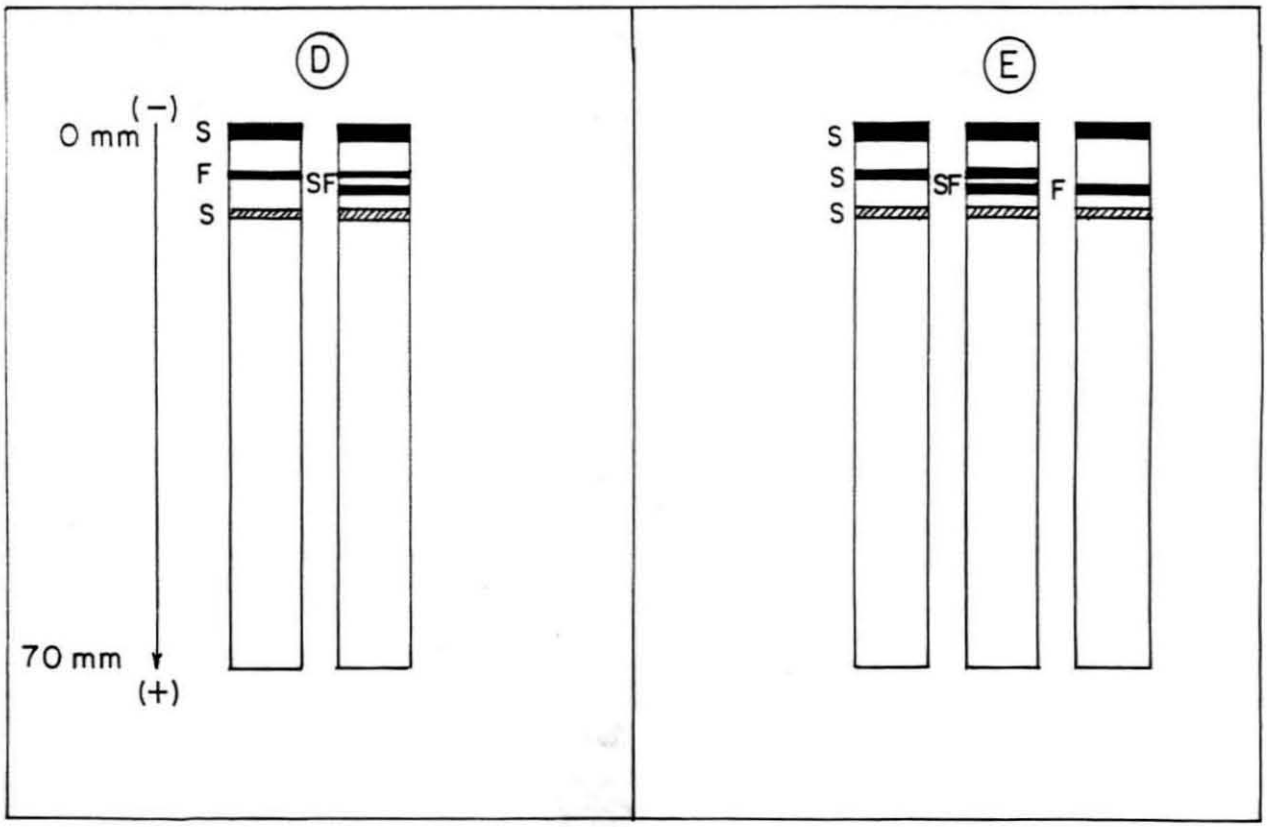
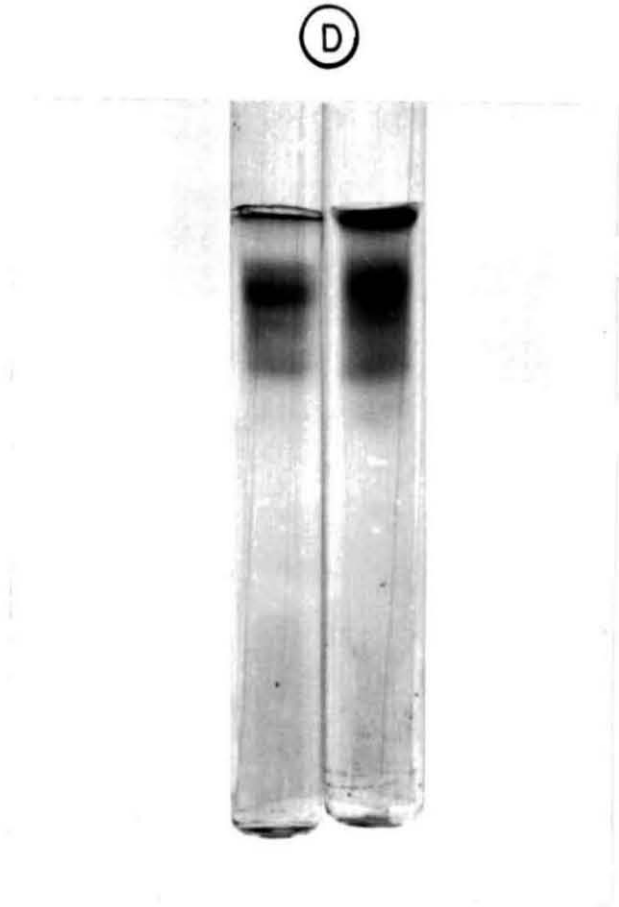


PLATE 4



type (**SF** band) heterozygotes were observed. But in Mandapam population slow type (**S** band) homozygotes and **SF** heterozygotes were observed and no fast homozygotes were present. In Madras population all the three phenotypes were observed. In all the populations the intensity of the enzyme was the same except for Mandapam population where it seemed to be little less in intensity (PLT. 4D). The bands were little diffused as seen in the photograph of Mandapam population. Similarly, in the Cochin population, the bands at AO-II locus were not so thick and broad as that of other populations, particularly the Calicut population. The third locus (AO-III) shown between 12 mm and 14 mm was polymorphic only in the Cochin population with homozygotes (**F** type) and heterozygotes (**SF** type).

The allele frequencies calculated for the three loci are tabulated in the Table 2. Since the AO-I locus was non-polymorphic in all the populations studied, the values are the same. The frequencies of the major allele **F** at II locus varied from 0.275 (Mandapam) to 0.687 (Cochin). However, its range was of lesser order between Cochin, Calicut, Mangalore and Madras, it being 0.50 to 0.69 (TBL.2). The Mandapam population distinguished itself with the domiant **S** allele having a frequency of 0.725. The nature of allele frequencies at AO-III locus is of special interest. Cochin population alone showed frequencies for both **S** and **F** alleles where **F** had a frequency of 0.81. Its frequency in Calicut and Mangalore was 1.00 whereas Mandapam and Madras possessed cent percent **S** allele. Significant differences in frequencies of specific allele at AO-III locus suggest that Cochin population is significantly different from Calicut and Mangalore in respect of **F** allele,

while it is more significantly different from Mandapam and Madras where cent percent **S** allele was present. Again Calicut and Mangalore populations are very different from Mandapam and Madras populations in having fixed **F** allele in the former group and fixed **S** allele in the latter group.

Values of observed and expected genotype frequencies in the sample populations are tabulated in the Table 3. Except for the Mandapam population, all the other stations showed deviations from Hardy-Weinberg equilibrium for AO-II locus due to excess of heterozygotes. In Mandapam population the expected values for **F** and **S** homozygotes are in good agreement. In Calicut and Mangalore only heterozygotes were present. Hence, the chi-sqare values are highly significant (TBL.9). The expected values for third locus of AO (AO-III), which was polymorphic only in Cochin showed agreement with the observed values (TBL.3).

4.1.3 ESTERASE (EST)

The zymogram patterns of esterase enzyme system of Sardinella longiceps collected from Cochin, Calicut, Mangalore, Mandapam and Madras are shown in Figures 5,6,7,8,9 and Plates 5,6,7,8,9. Based on intensity of enzyme activity, the patterns can be differentiated into a zone of darkly stained thick bands and lightly stained narrow bands with the exception, where one of the bands was also appeared to be darker as in Cochin population (PLT.5). A close comparison of the bands at darkly stained zone shows differences in the staining intensity as well as size of the bands in different populations. However, based on band combinations at a particular zone, the patterns can be grouped into four presumed loci. The first two loci

FIGURE 5 AND PLATE 5

Zymogram patterns of Esterase in

S. longiceps populations from

	Locus		
A, A_1 - Cochin	I	=	0-5 mm
	II	=	12-15 mm
	III	=	21-26 mm
	IV	=	33-40 mm

FIG. 5

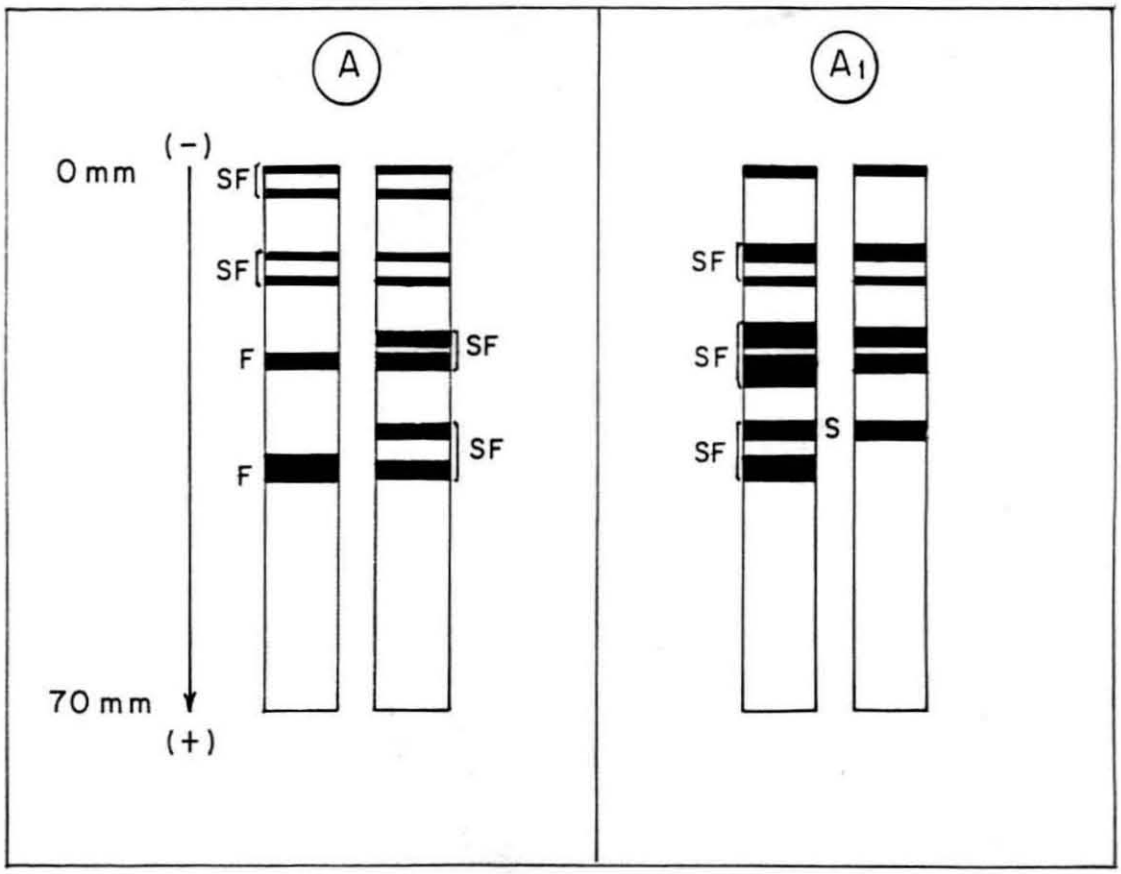
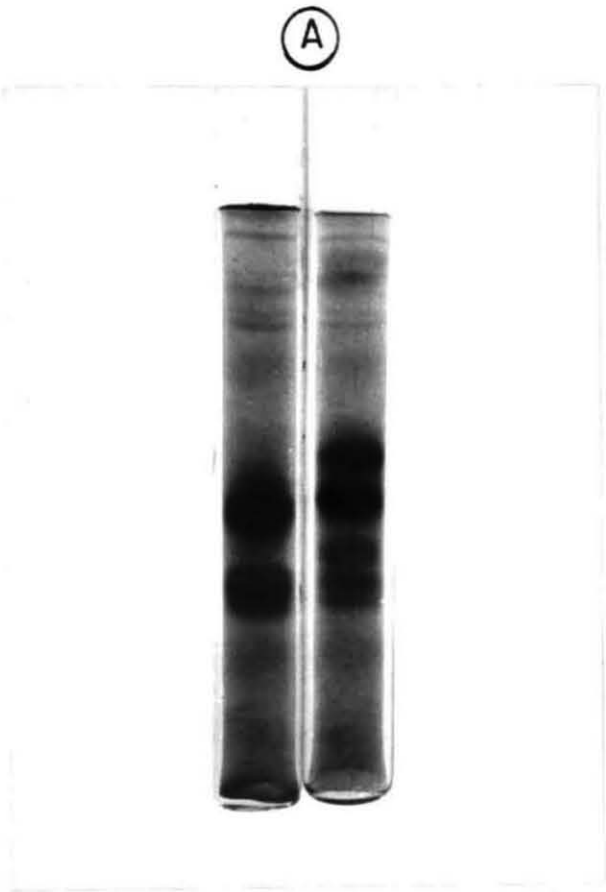


PLATE 5



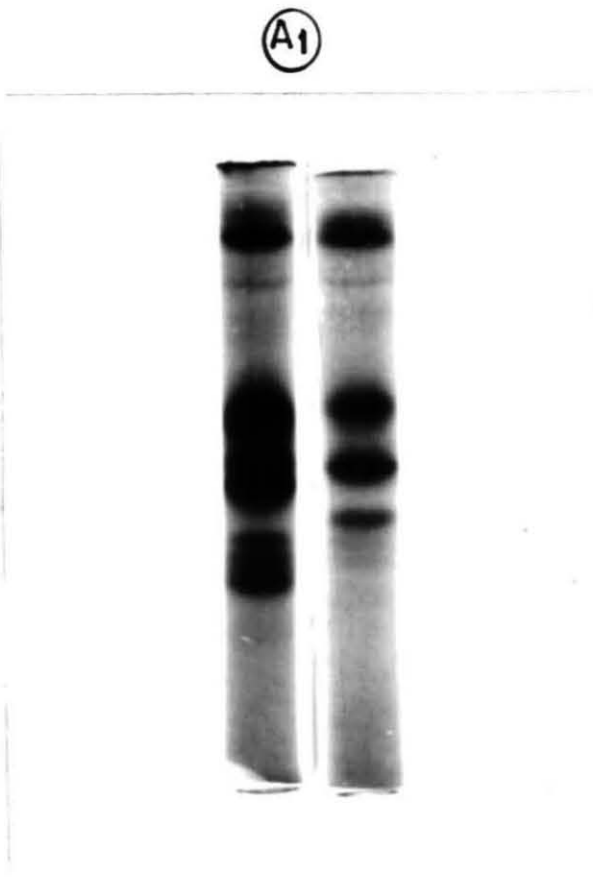


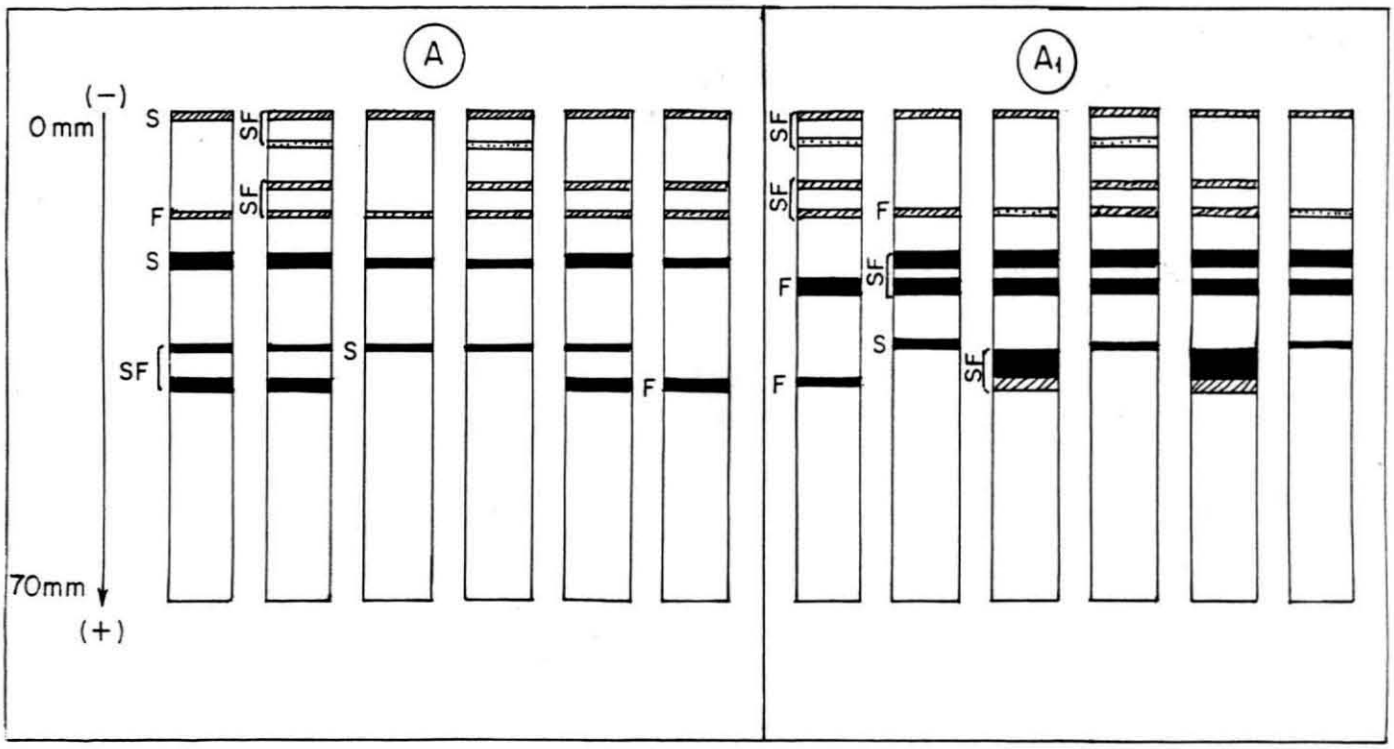
FIGURE 6 AND PLATE 6

Zymogram patterns of Esterase in

S. longiceps populations from

	Locus		
A, A_1, A_2 - Calicut	I	= 0-5	mm
	II	= 12-15	mm
	III	= 21-26	mm
	IV	= 33-40	mm

FIG. 6


A
(−)
0mm
S
SF
SF
F
S
SF
S
F
70mm
(+)
A1
SF
SF
F
F
SF
S
SF

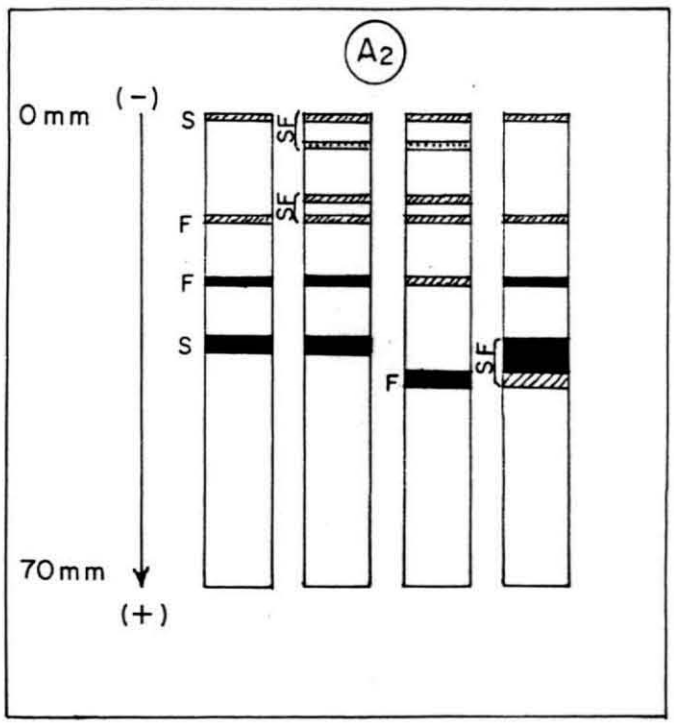

A2
0mm
(−)
S
SF
SF
F
F
S
F
SF
70mm
(+)

PLATE 6

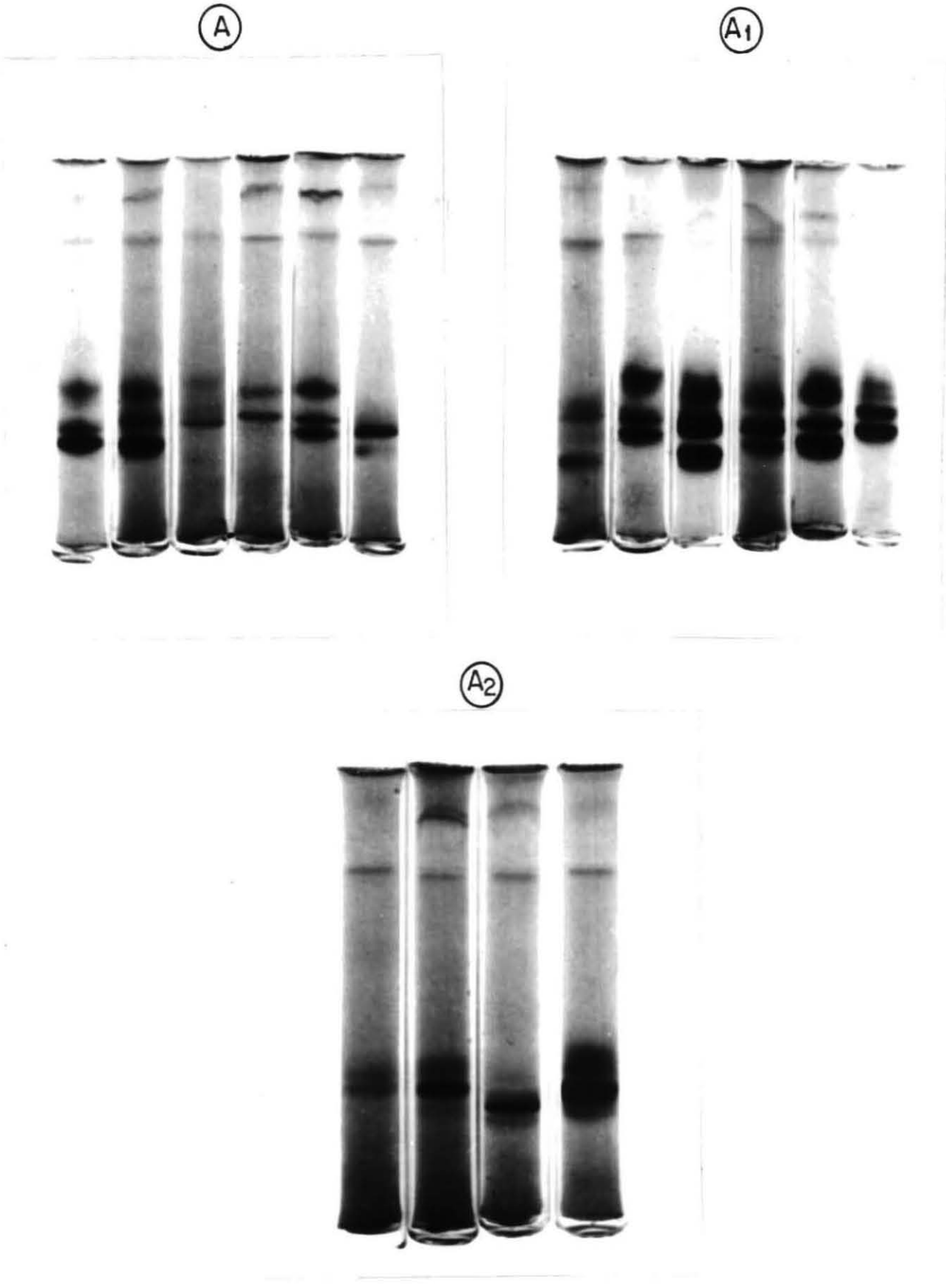


FIGURE 7 AND PLATE 7

Zymogram patterns of Esterase

in S. longiceps populations from

		Locus		
A, A_1 - Mangalore	I	=	0-5	mm
	II	=	12-15	mm
	III	=	21-26	mm
	IV	=	33-40	mm

FIG. 7

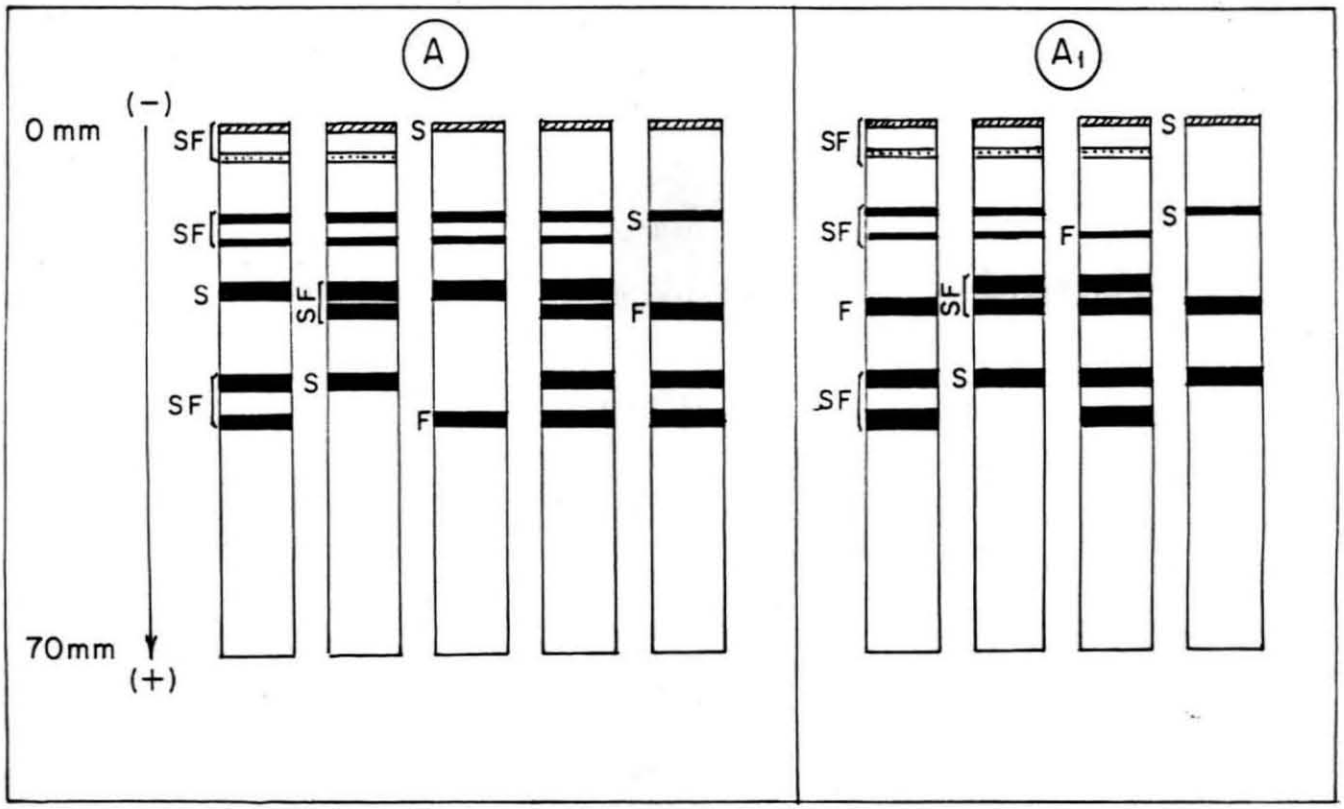
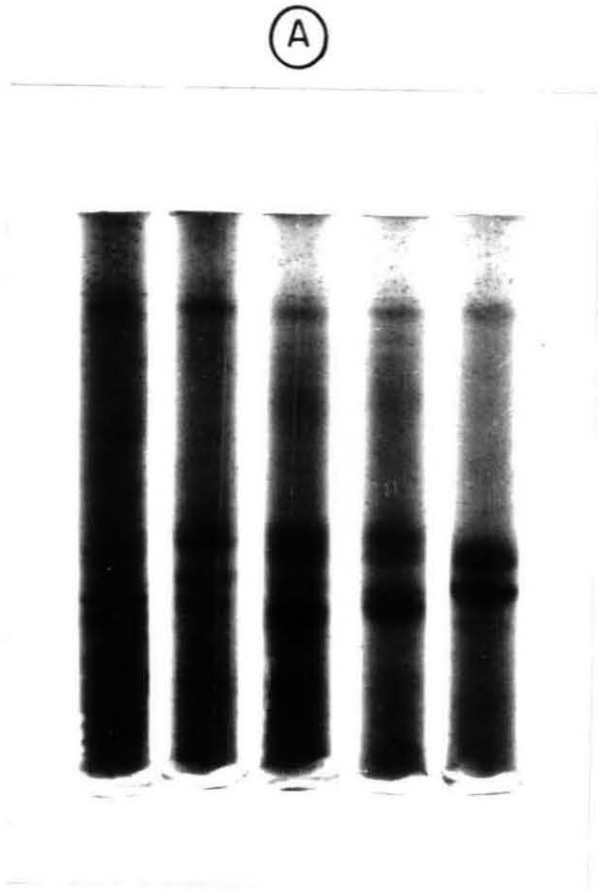


PLATE 7



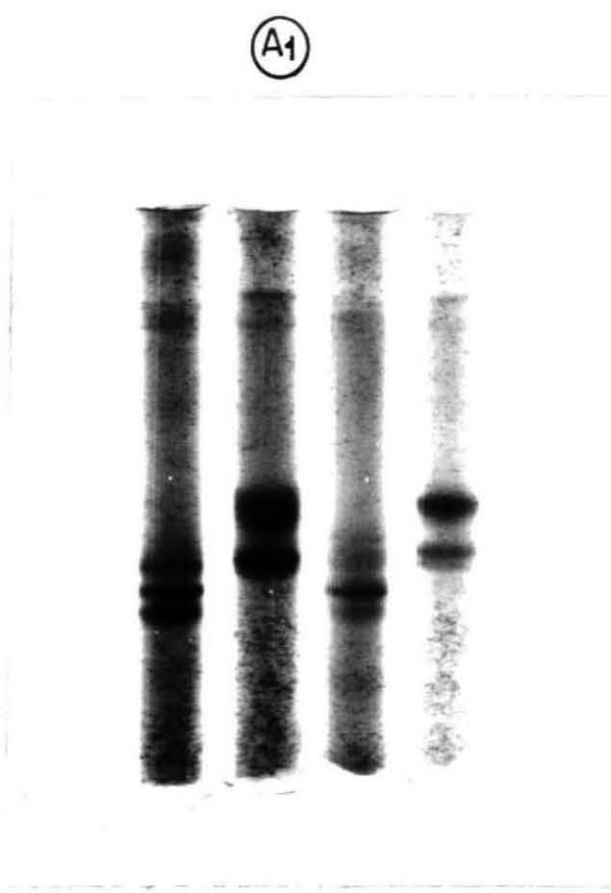


FIGURE 8 AND PLATE 8

Zymogram patterns of Esterase in

S. longiceps populations from

		Locus		
A - Mandapam	I	=	0-5	mm
	II	=	12-15	mm
	III	=	21-26	mm
	IV	=	33-40	mm

FIG. 8

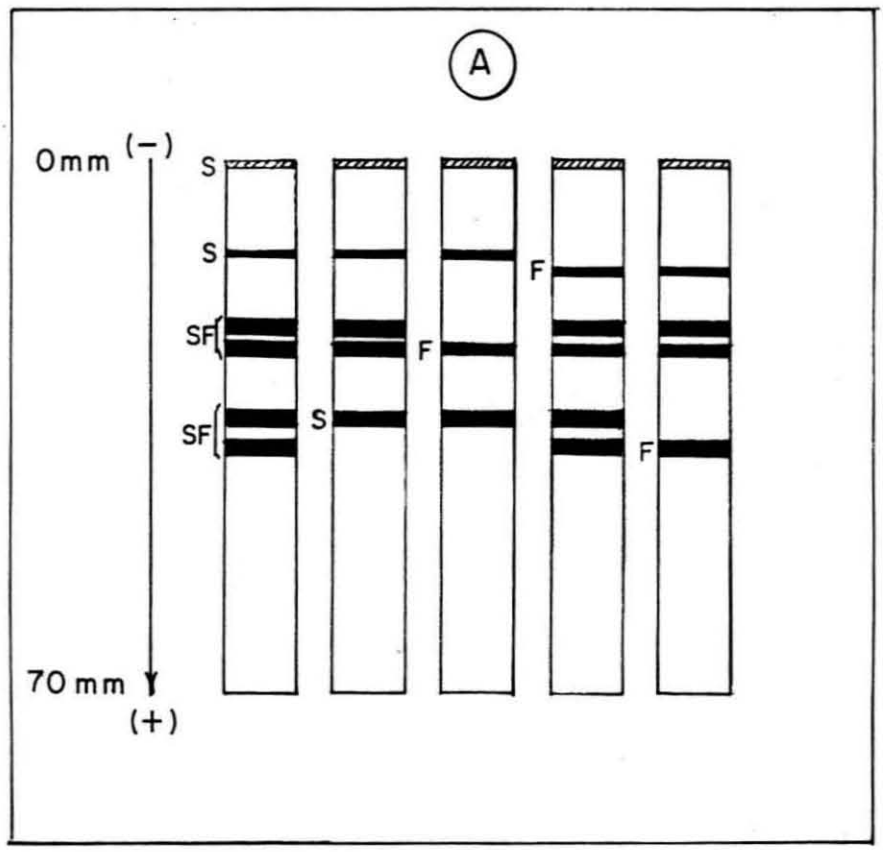


PLATE 8

(A)

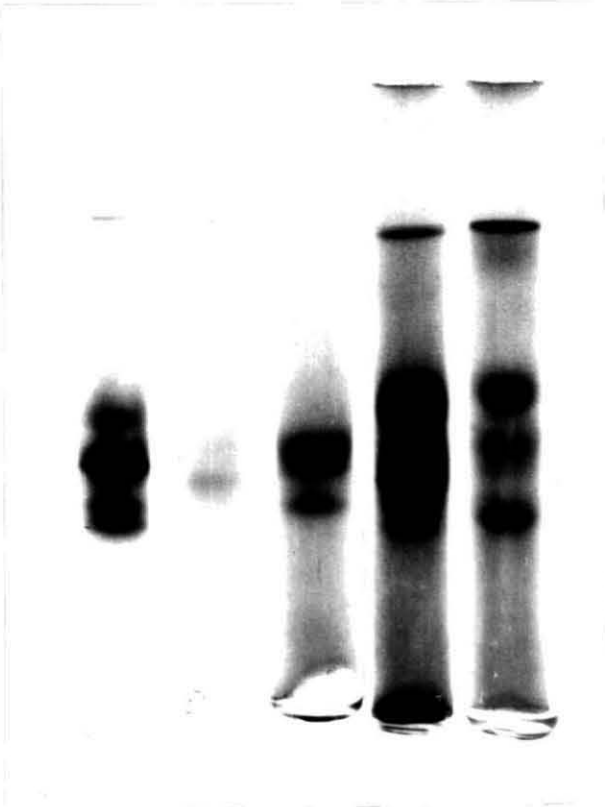


FIGURE 9 AND PLATE 9

Zymogram patterns of Esterase in

S. longiceps populations from

	Locus		
A, A_1 - Madras	I	=	0-5 mm
	II	=	12-15 mm
	III	=	21-26 mm
	IV	=	33-40 mm

FIG. 9

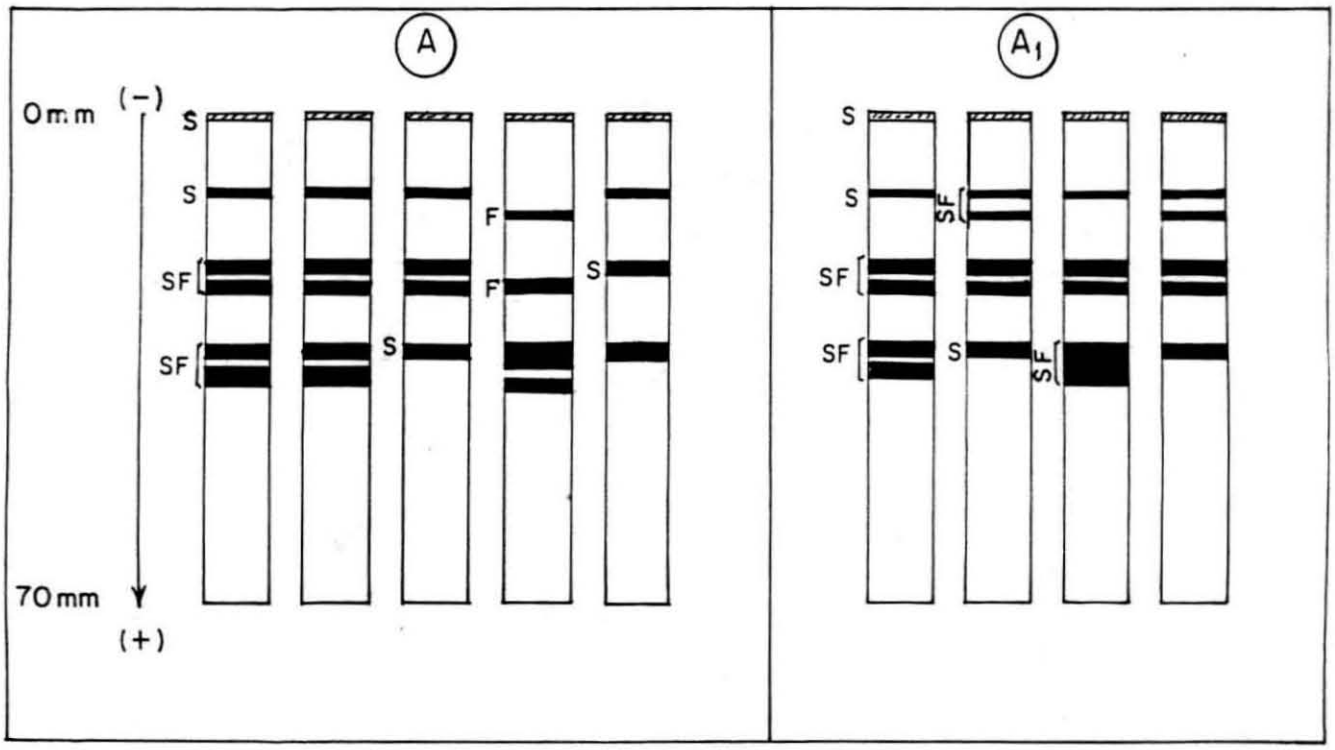
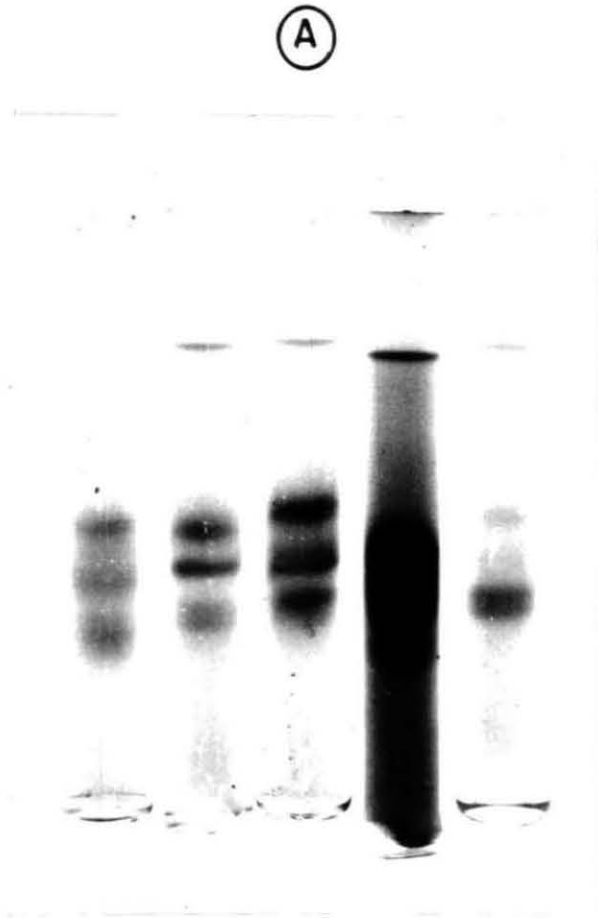
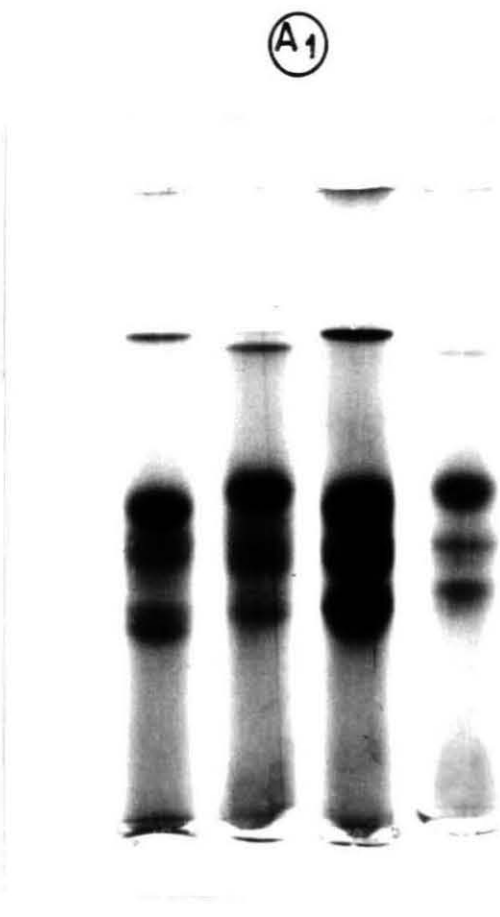


PLATE 9





consisted of lightly stained narrow bands. Thus the activity of first locus appeared to be restricted to the top gel area. The lightly stained narrow bands of second locus occured between the first and third locus. The dark thick bands of third and fourth loci were found moving closely and much ahead of the lightly stained first and second loci. A general comparison of band patterns in each locus in all the populations indicated that the esterase enzyme system in S. longiceps is polymorphic. The variant phenotype patterns of first locus consisted of single and double banded types at Cochin, Calicut and Mangalore whereas, Mandapam and Madras populations showed only a single banded phenotype. The phenotype patterns of second locus in each population were single and double banded. Depending on the mobility of these single and double banded phenotypes, they were designated as slow moving **S**, fast moving **F** and a combination of these two was **SF**. Similarly, an overall comparison of band positions at third and fourth loci showed again their polymorphic nature in the species. The heterozygous phenotype patterns at third and fourth loci were two banded as in the case of first and second loci. However, heterozygous types from Cochin appeared to show triple banded condition (FIG.5). The populations from Mandapam and Madras showed only single banded similar phenotypes at the third locus. These three phenotypes at III and IV EST loci were again designated as **S, F** and **SF** as described in the case of first and second loci.

The Table 2 gives allele frequencies at all the four loci in different populations tested. The frequency values of the presumed **S** and **F** alleles at first locus indicate **S** as the predominant allele in all the populations.

However, its value was significantly different in populations from Mandapam and Madras, it being cent percent in these two populations, whereas it ranged from 0.73 (Calicut) to 0.93 (Mangalore) in other populations. The frequency values of **S** and **F** alleles at the second locus in almost all the populations differed in one or other respect. The **S** allele appeared to be predominant in all the populations except Calicut population where it was **F** allele. The frequencies of **S** and **F** alleles at third locus in Cochin and Mandapam also considerably differed from that of Calicut, Mangalore and Madras populations. The predominant alleles were different in these two groups. The allelic frequencies at third locus were significantly different between Madras and Mandapam. On the other hand the frequency values of **F** and **S** alleles of Calicut, Mangalore and Madras populations were closely comparable. Though the predominant allele in all the populations at fourth locus is **S**, its value was considerably much higher at Mangalore than that of other regions. An overall comparison of frequency values of **S** and **F** alleles at all the four loci thus showed significant East-West regional as well as inter populational differences with in each region.

Table 3 presents observed and expected esterase genotype frequencies as well as chi-squared values. The values significantly deviated only in three occasions out of 20. The significant deviations occured twice in Cochin at Second and third loci and once in Madras at second locus. Excess of heterozygotes in Cochin and homozygotes in Madras produced the deviation.

4.1.4 GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD)

Zymogram patterns of Glucose-6-phosphate dehydrogenase (G6PD) in Sardinella longiceps observed from Cochin, Calicut, Mangalore, Mandapam and Madras are shown in Figure 10,11 and Plate 10,11. The zymogram showed five zones of activity which were attributed to five loci. These zones were identified according to mobility differences of a band or set of bands. The top of the gel was having an invariant band (0 mm) which was designated as G6PD-I locus. In all the five stations studied the first locus was non-polymorphic. In Cochin population the activity of first locus was less when compared with other centres which showed 3x intensity (FIG.10A; PLT.10A). Another invariant band at 3 mm was observed in all regions. This was designated as second locus (G6PD-II). All the different populations sampled showed an intensity of 1x except Calicut population, where the band showed 2x activity in some animals and 1x activity in other animals. The second tube of Calicut population's photograph shows this increased activity (FIG.10B; PLT.10B).

Unlike the first and second non-polymorphic loci, the G6PD-III locus was polymorphic in nature. Out of the five populations sampled, Mandapam and Madras were showing bands at 7 mm and 8 mm. However, Cochin, Calicut and Madras showed a single band at 7 mm suggesting its homozygous nature with reference to double band heterozygous type at 7 mm and 8 mm present in other two populations. Single banded **F** homozygotes and double banded **SF** heterozygotes were observed in Mandapam and Madras populations. But single banded **S** homozygotes were totally absent. In addition to these three loci, all the five stations also showed bands at 13 mm and 15 mm positions. These invariant bands were designated as fourth (G6PD-IV) and

FIGURE 10 AND PLATE 10

Zymogram patterns of Glucose-6 phosphate Dehydrogenase in S. longiceps populations from

		Locus		
A -	Cochin	I	= 0-1	mm
B -	Calicut	II	= 3	mm
C -	Mangalore	III	= 6-8	mm
		IV	= 12-13	mm
		V	= 14-15	mm

FIG. 10

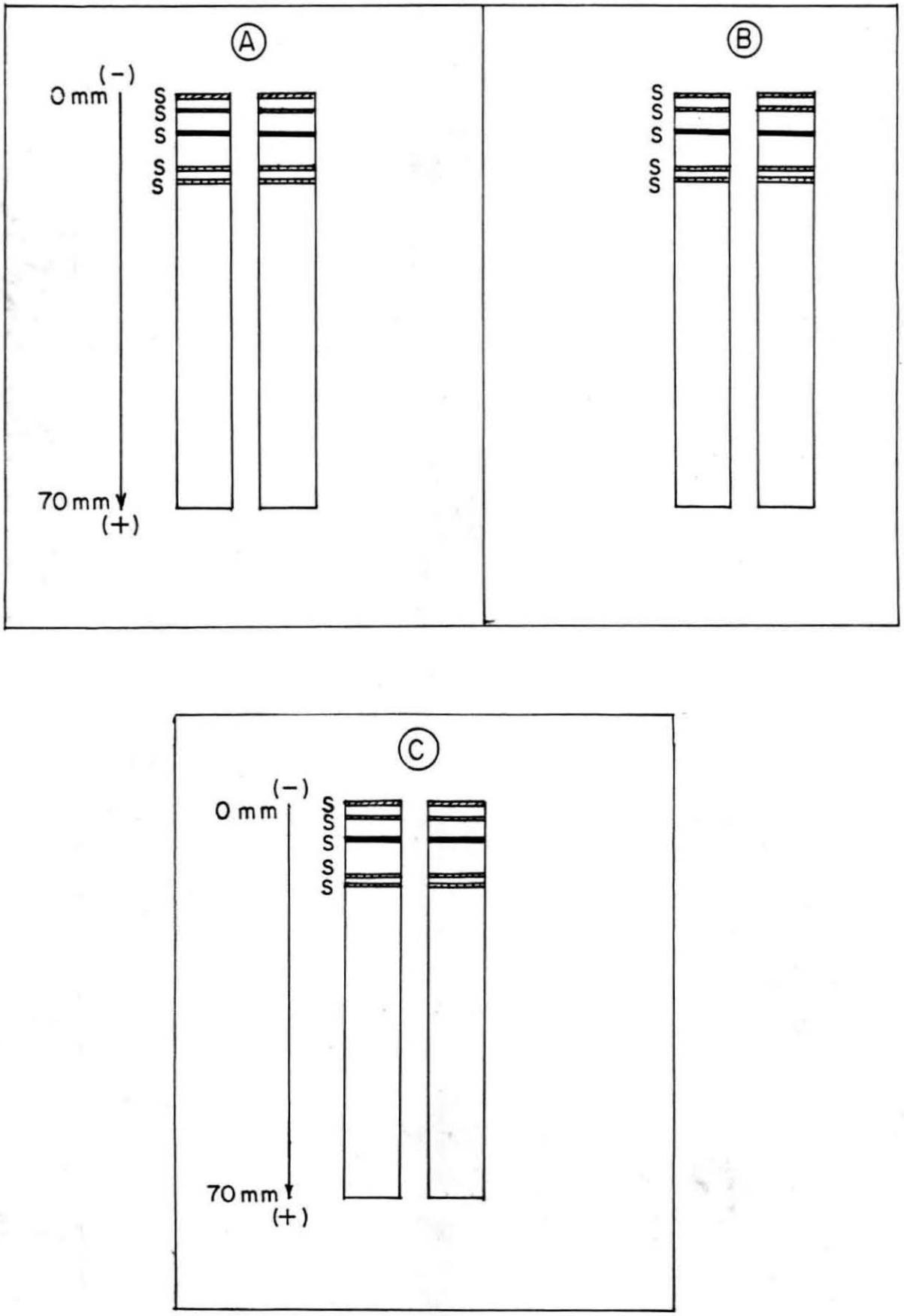
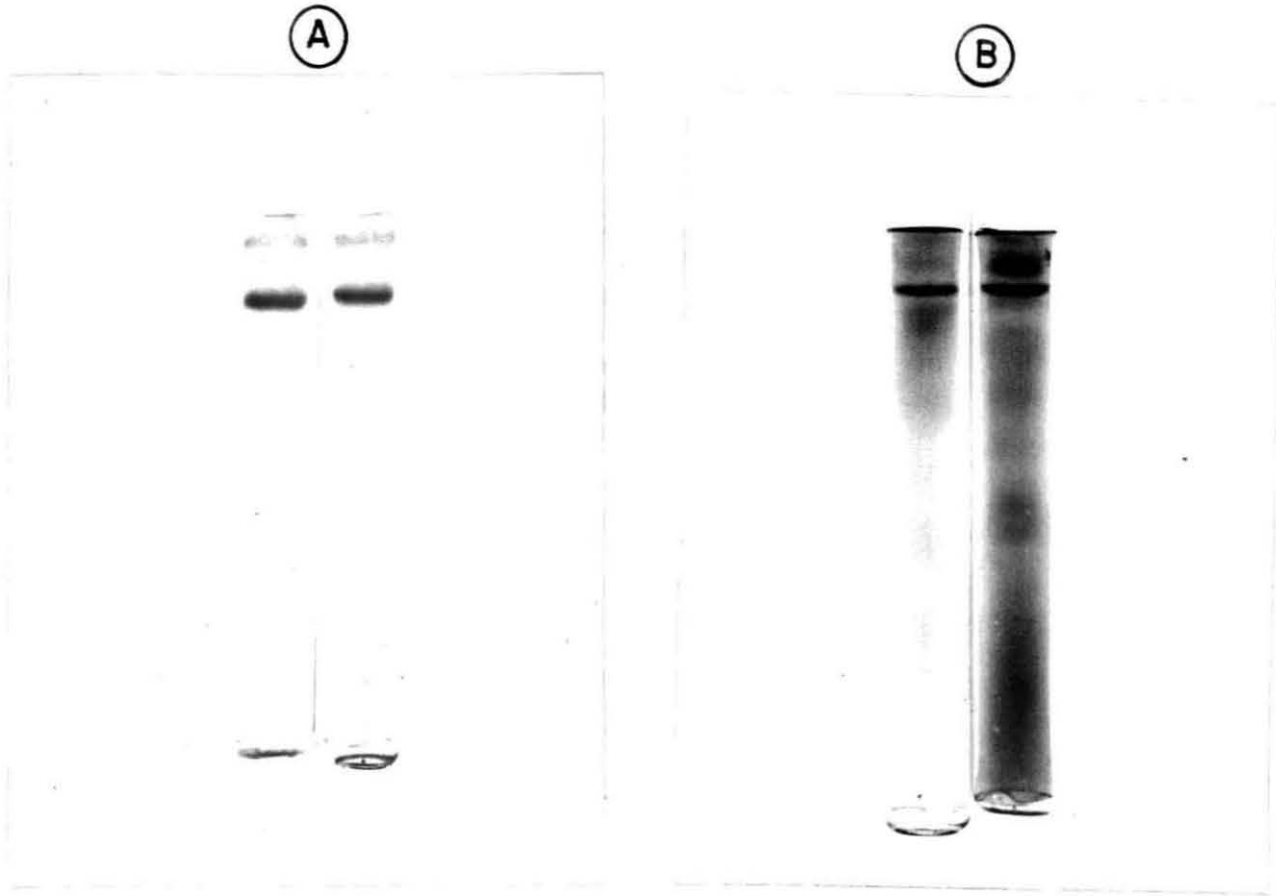


PLATE 10



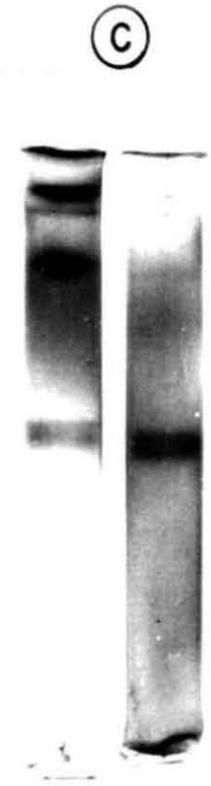


FIGURE 11 AND PLATE 11

Zymogram patterns of Glucose-6-Phosphate Dehydrogenase in S. longiceps populations from

D - Mandapam
E - Madras

Locus

I	=	0-1	mm
II	=	3	mm
III	=	6-8	mm
IV	=	12-13	mm
V	=	14-15	mm

FIG. 11

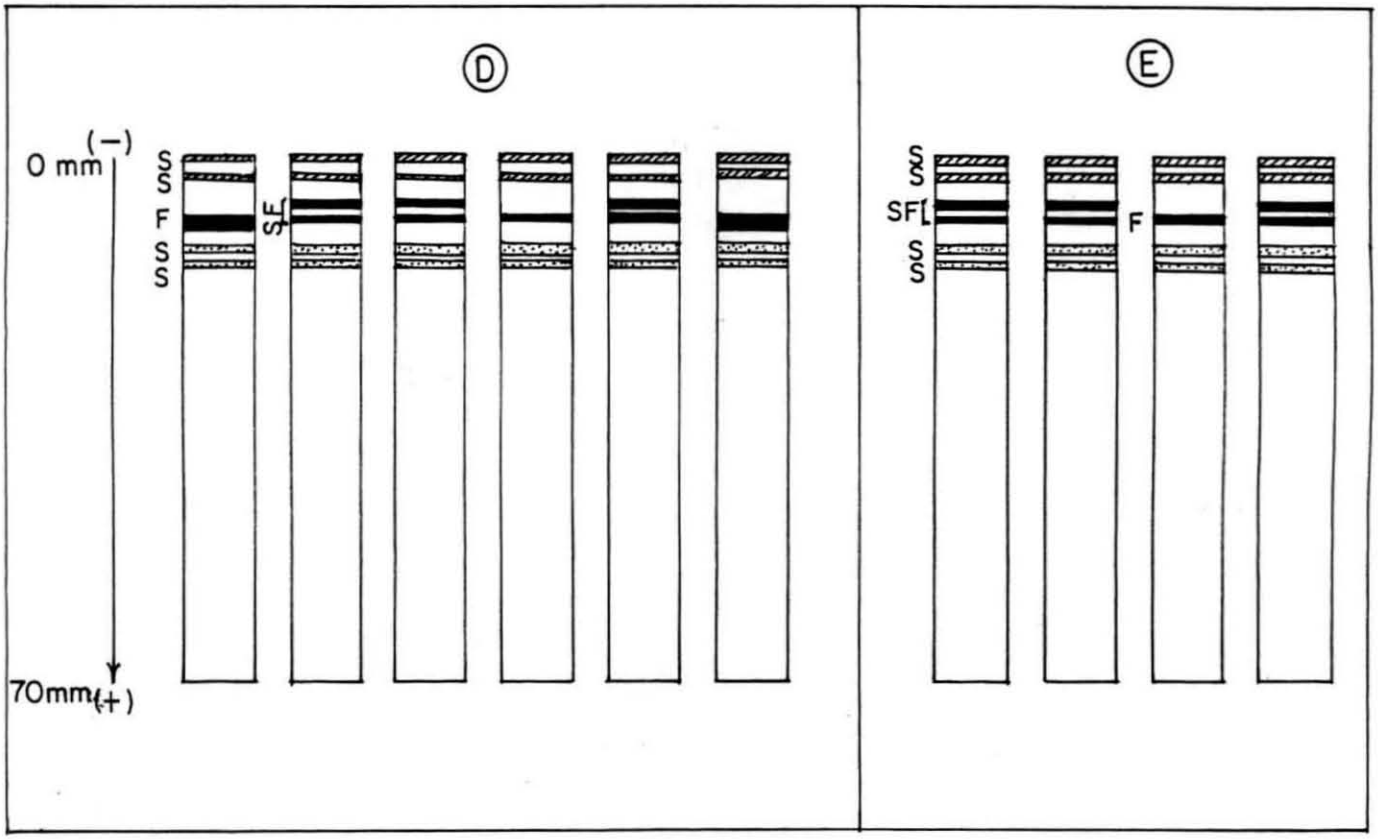
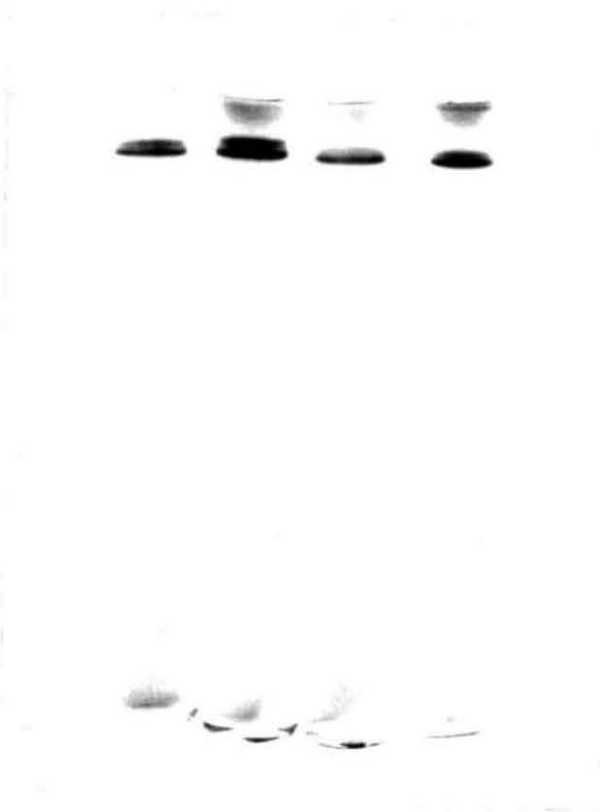


PLATE 11

(D)

(E)



fifth (G6PD-V) loci respectively. Since these bands did not show any variation, G6PD-IV and V loci were considered as non-polymorphic in the five populations sampled. The absence of polymorphism at all the five loci of G6PD in West coast populations and polymorphism at III loci in East coast populations clearly suggests that S. longiceps of East-West coast of India are genetically very different.

Allelic frequencies calculated for different loci are shown in Table 2. Allelic frequencies at G6PD I, II, IV and V show cent percent **S** allele (**S** = 1.00) in all the five populations. In case of G6PD-III locus, Cochin, Calicut and Mangalore populations were monomorphic, the allele frequency of **S** being 1.00. But in Mandapam and Madras populations the frequency of **F** allele was more, it being 0.76 for Mandapam and 0.53 for Madras. Madras and Mandapam populations also appeared to possess different frequencies at the third locus. The differences in the G6PD allelic frequencies of the populations of West coast (Cochin, Calicut and Mangalore) and that of East coast (Madras and Mandapam) are significant.

The observed and expected values of genotype frequencies are shown in Table 3. In Mandapam population, the observed and expected values for G6PD-III locus were in agreement with the Hardy-Weinberg proportions. In contrast the Madras population showed significant deviation from the Hardy-Weinberg equilibrium, due to excess of heterozygotes (TBL.3).

4.1.5 GLUTAMATE DEHYDROGENASE (GDH):

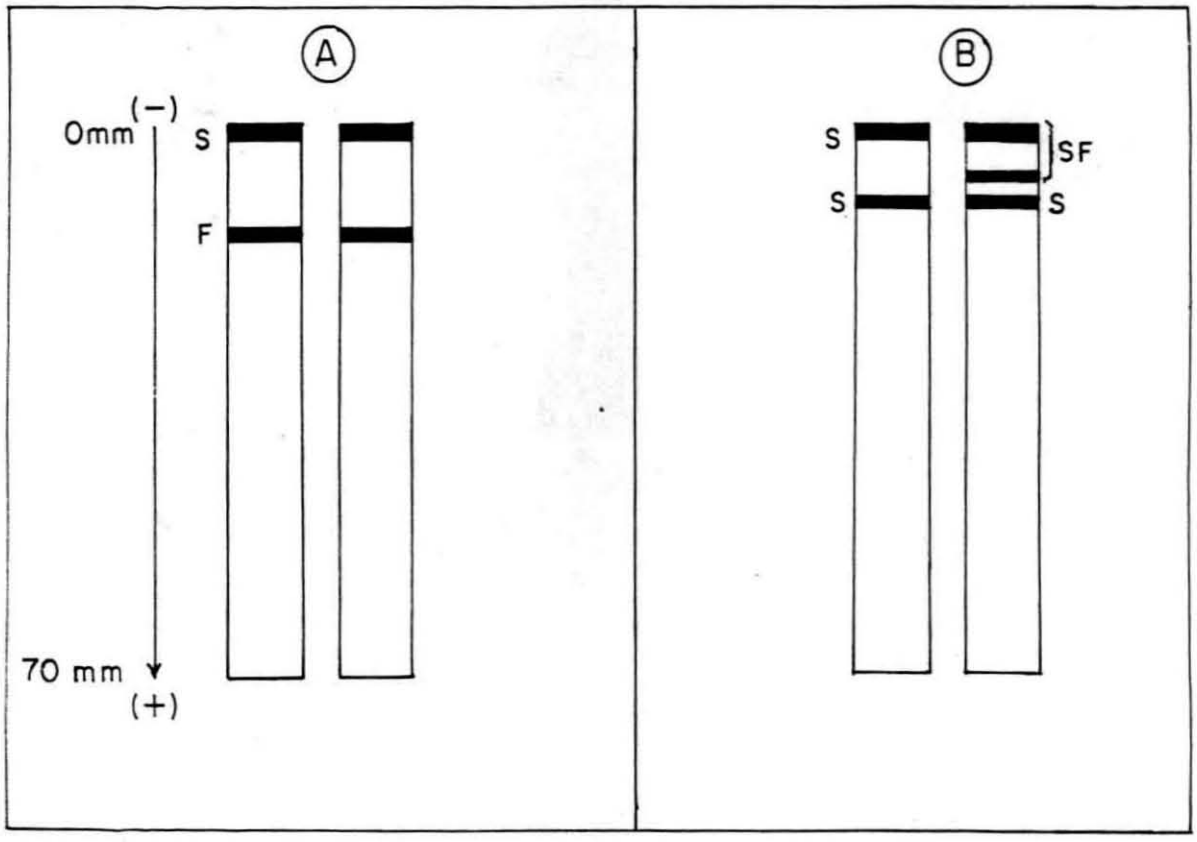
Zymogram patterns of GDH enzyme in populations of Sardinella longiceps studied from Cochin, Calicut, Mangalore, Mandapam and Madras

FIGURE 12 AND PLATE 12

Zymogram patterns of Glutamate Dehydrogenase in S. longiceps populations from

		Locus		
A -	Cochin	I	= 0-7	mm
B -	Calicut	II	= 9-15	mm
C -	Mangalore			

FIG. 12



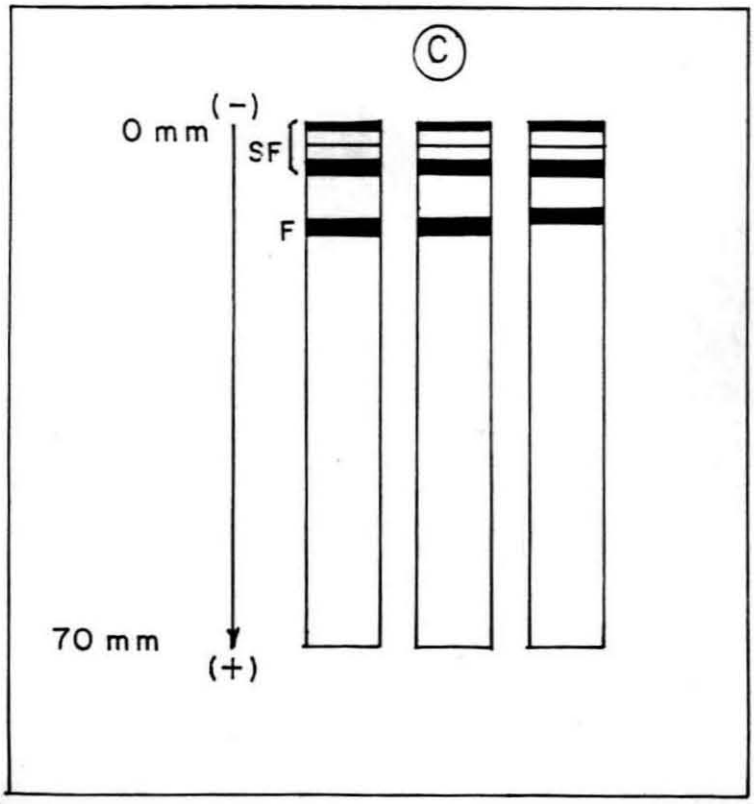


PLATE 12

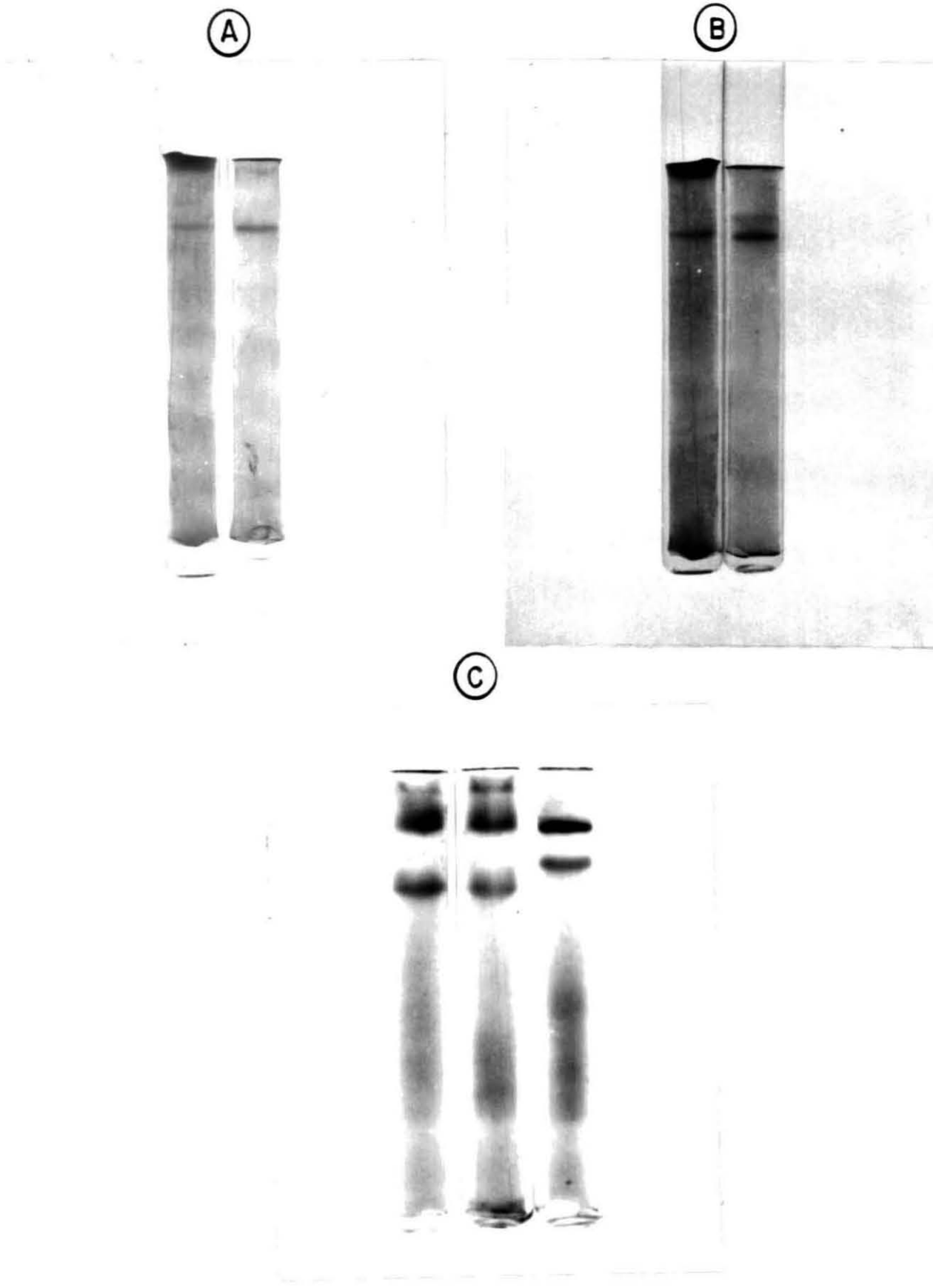


FIGURE 13 AND PLATE 13

Zymogram patterns of Glutamate Dehydrogenase

in S. longiceps populations from

		Locus		
D -	Mandapam	I	=	0-7 mm
E -	Madras	II	=	9-15 mm

FIG. 13

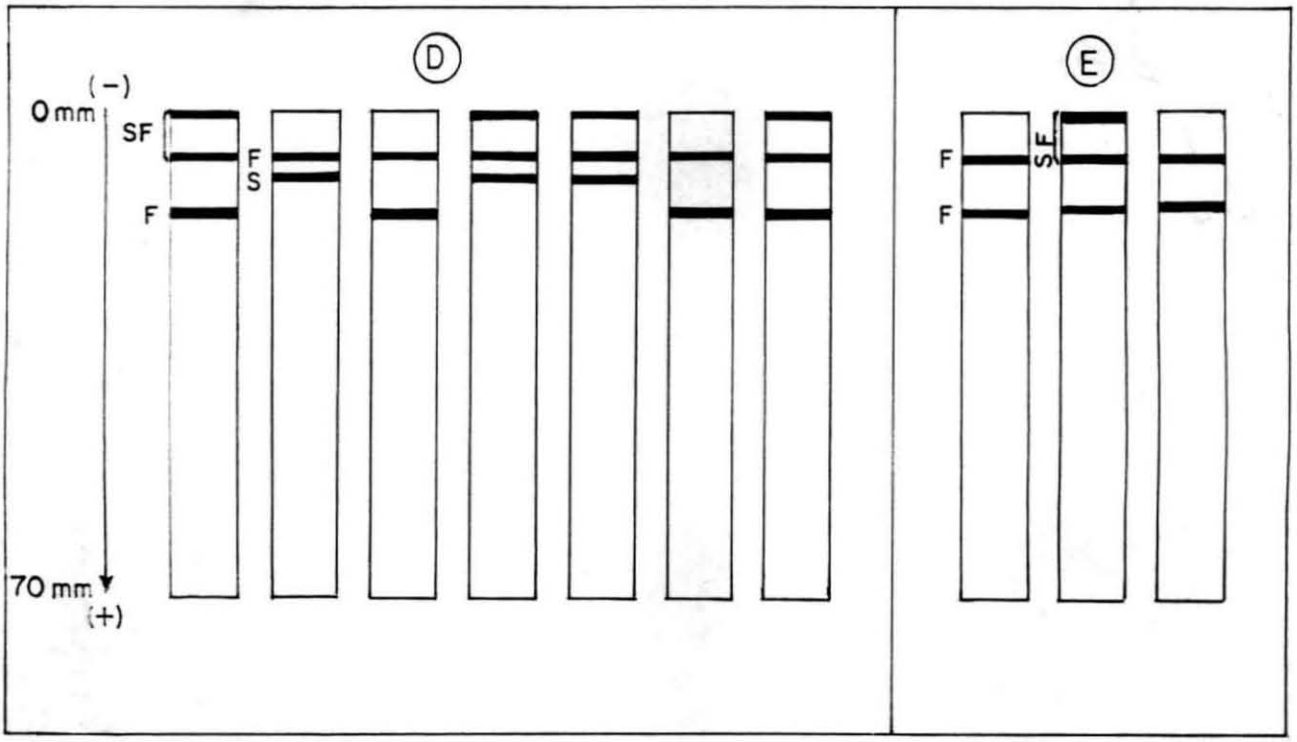
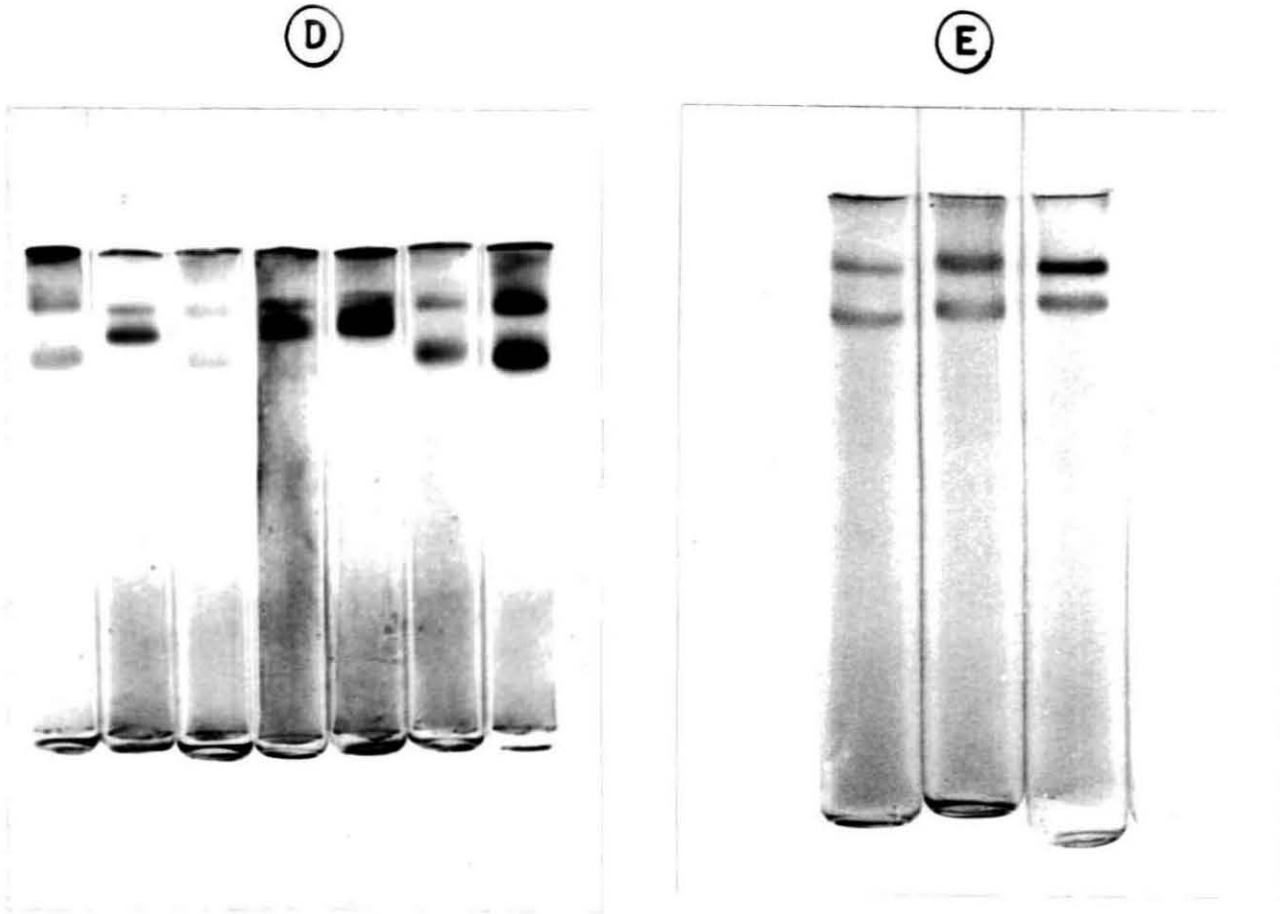


PLATE 13



are shown in Figure 12,13 and Plate 12,13. The zymogram showed two different zones of enzyme activity which are presumed to be under the control of two loci. They are designated as GDH-I and GDH-II according to their order of increasing mobility. Activity of GDH-I locus occured between 0-7 mm gel position. The activity of the II locus, GDH-II was found between 9 mm and 15 mm. Both GDH-I and II loci showed phenotype variation. The polymorphism at GDH-I locus at Cochin produced slow type (**S** band) and fast type (**F** band) homozygotes and slow-fast type (**SF** band) heterozygotes. However, in Mangalore, Mandapam and Madras populations only fast homozygotes and heterozygotes were present, whereas in Calicut population slow homozygote and heterozygote were found. The mobility of GDH-I fast band in Calicut and Madras populations was slightly more than that of Cochin, Mangalore and Mandapam population (PLT.12B and 13E). Interestingly, the mobility of GDH-II slow band seemed to be more slower in Mangalore and Mandapam populations (PLT. 12C and 13D). Also the bands in general were narrow in Calicut and Madras populations (PLT.12B and 13E). Unlike GDH-I which is polymorphic in all populations studied, the GDH-II showed polymorphism in Calicut and Mandapam only. Besides, while cent percent **F** allele was observed in Cochin, Mangalore and Madras populations, Calicut population showed cent percent **S** allele; whereas Mandapam population showed slow homozygote and fast homozygote but no heterozygotes were observed.

The allelic frequencies of Glutamate dehydrogenase first and second loci are summarised in the Table 2. In Cochin, Mangalore, Mandapam and Madras populations the **F** allele was found to be the major allele at GDH-

I locus. The frequency of **F** allele ranged from 0.54 at Mangalore to 0.90 at Madras. In contrast to this in Calicut population the **S** allele was the major allele (**S**=0.76). As regard to GDH-II locus **F** was the major allele in Cochin, Mangalore and Madras populations (**F**=1.00), whereas Calicut showed cent percent **S** allele (**S**=1.00). However, in Mandapam population **S** allele frequency was 0.68 and **F** allele frequency was 0.32. Thus significant differences in the GDH allelic frequencies between local populations as well as regional populations suggest that populations of S. longiceps from Cochin, Calicut, Mangalore, Mandapam and Madras are genetically different.

Values of observed and expected genotype frequencies in the sample populations are tabulated in the Table 3. Cochin and Mangalore populations showed significant deviations from the Hardy-Weinberg equilibrium whereas observed values of Calicut, Mandapam and Madras showed good agreement with the expected values. In both Cochin and Mangalore the disagreement was due to the excess of heterozygotes at GDH-I locus. The expected genotype values in Mandapam population was also deviated due to excess of homozygotes.

4.1.6 ISOCITRATE DEHYDROGENASE (IDH)

Zymogram patterns of IDH enzyme in Sardinella longiceps populations studied from Cochin, Calicut, Mangalore, Mandapam and Madras are shown in Figure 14,15 and Plate 14,15. The zymogram patterns of all the five regions examined showed IDH enzyme activity in two different areas, the first at the top of the gel (0-1 mm) was designated as first locus (IDH-I) and the second observed in between the 5 and 9 mm was designated as

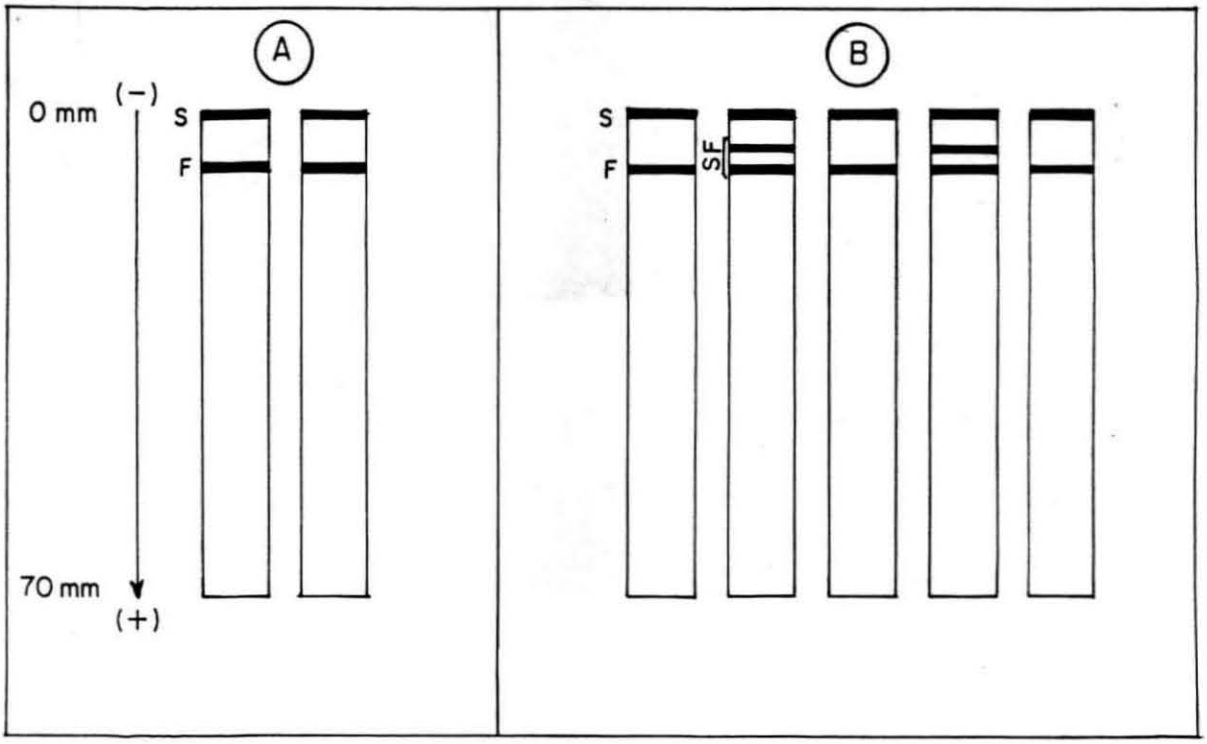
FIGURE 14 AND PLATE 14

Zymogram patterns of Isocitrate Dehydrogenase in

S. longiceps populations from

		Locus			
A -	Cochin	I	=	0-1	mm
B -	Calicut	II	-	5-9	mm
C -	Mangalore				

FIG. 14



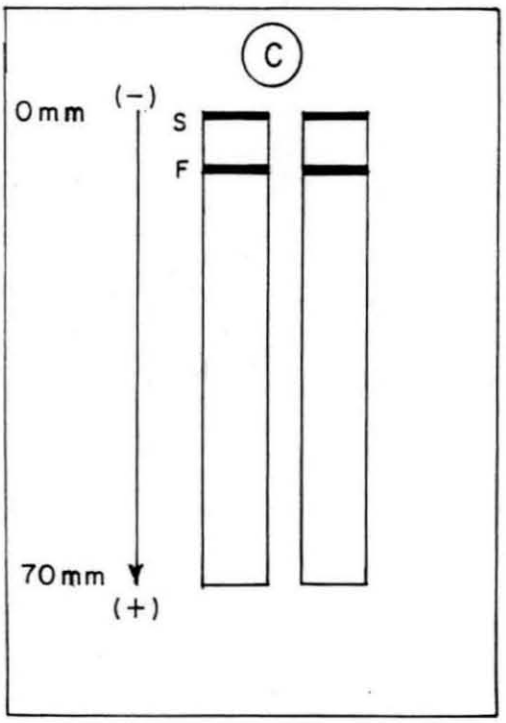


PLATE 14

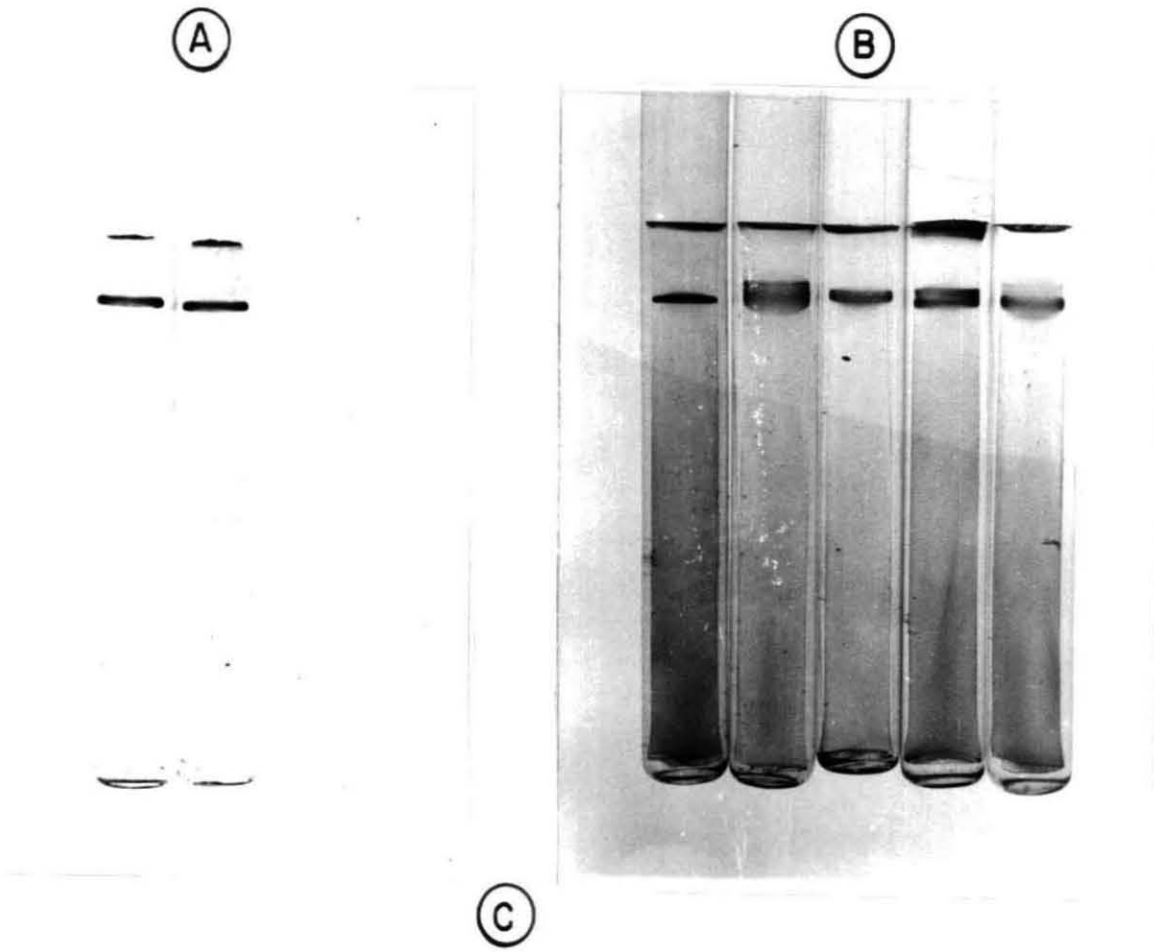


FIGURE 15 AND PLATE 15

Zymogram patterns of Isocitrate Dehydrogenase in

S. longiceps populations from

		Locus			
D -	Mandapam	I	=	0-1	mm
E -	Madras	II	=	5-9	mm

FIG. 15

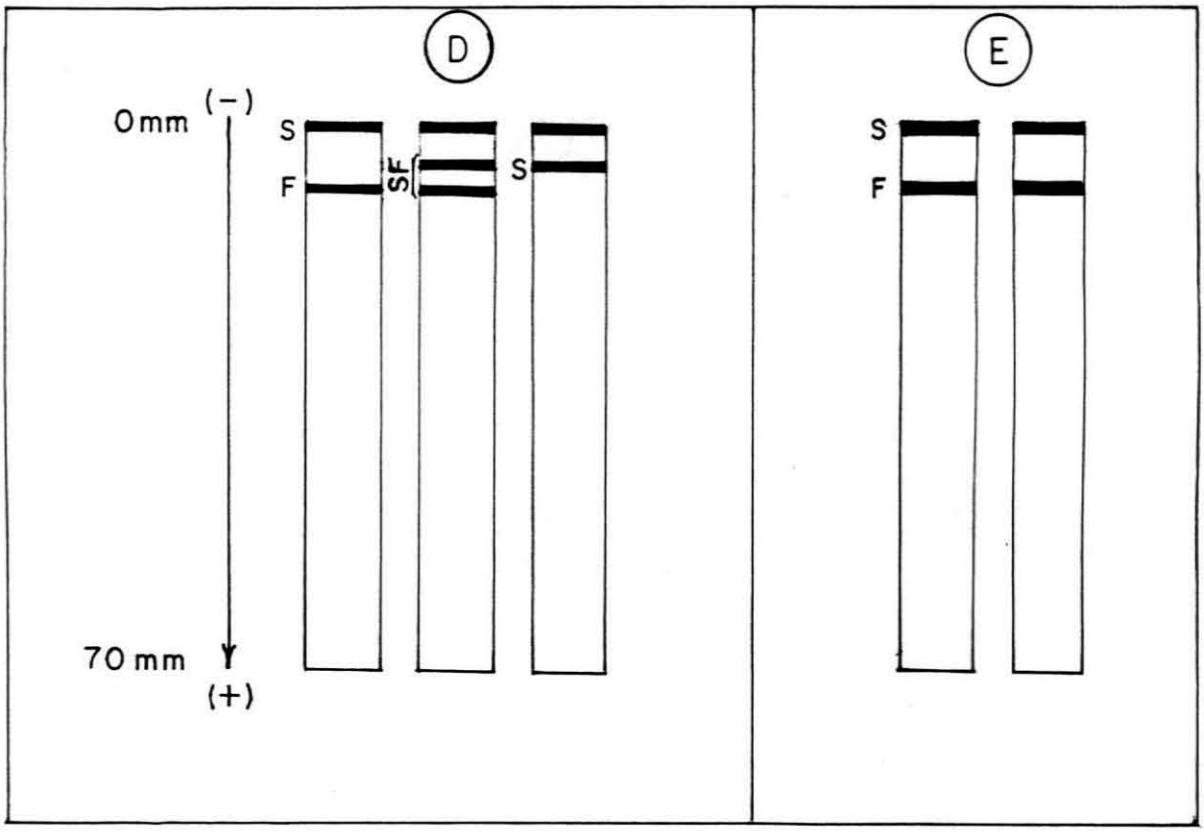
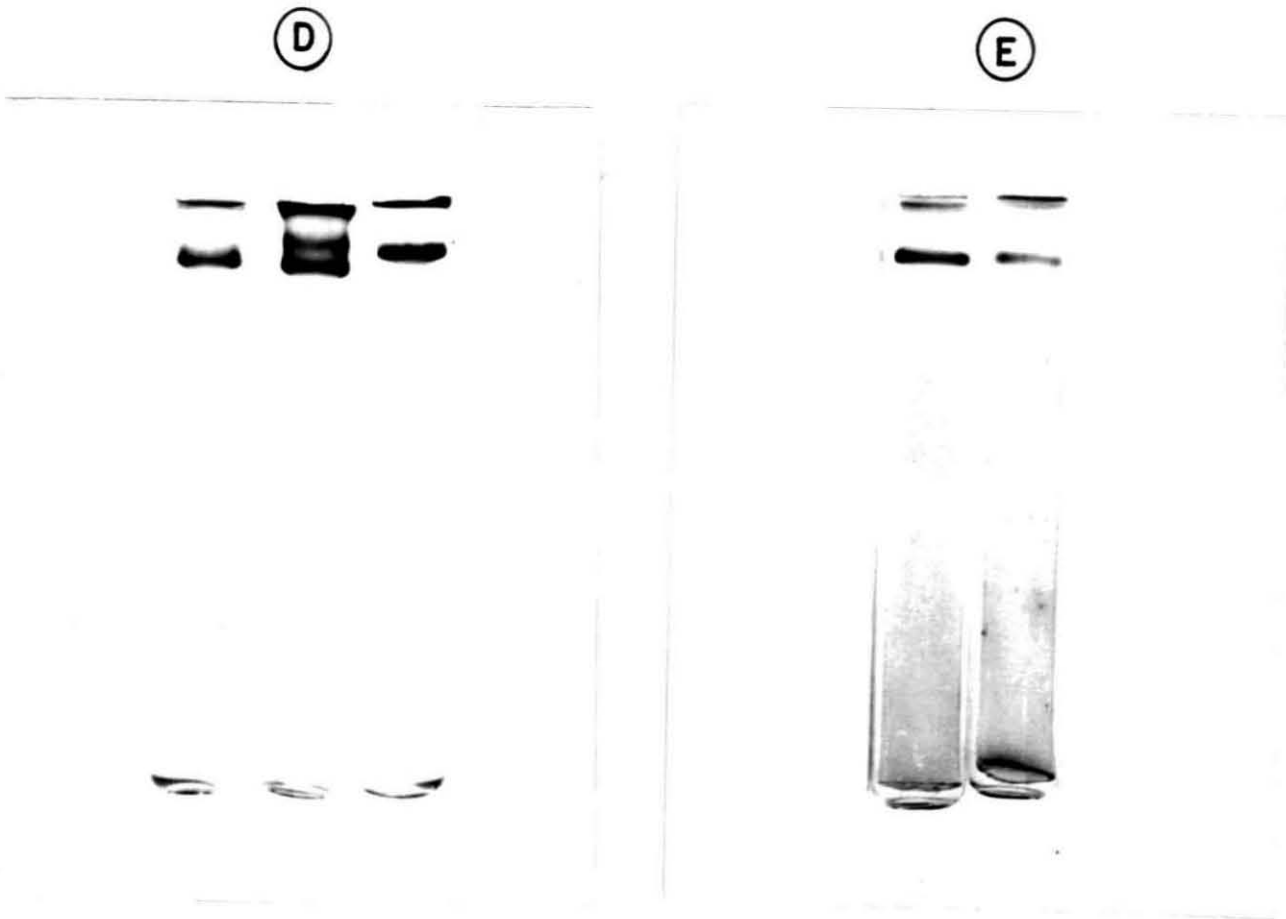


PLATE 15



second locus (IDH-II). In all the five populations studied IDH-I locus showed an invariant band. The enzyme activity of Cochin population appeared to be of lesser intensity than that of other areas (PLT.14A). The second locus was polymorphic in both Calicut and Mandapam populations, whereas it was non-polymorphic in Cochin, Mangalore and Madras populations, where only a single fast band **F** (8 mm) was observed. In Mangalore and Madras populations, eventhough the **F** band showed same activity (3x) in all the individuals tested, some animals showed slightly higher intensity (PLT.14C and 15E). The polymorphic II locus in Calicut and Mandapam populations showed three phenotypes. They are (1) a single banded slow homozygote **(S)** (2) double banded slow-fast **(SF)** heterozygote and a single banded fast homozygote **(F).** A close comparison of these three phenotype variants suggests that they may be co-dominant di-allele products at a single locus. The **S** and F bands of IDH-II locus were found at 7th mm and 8th mm respectively.

The allelic frequencies, observed and expected genotype frequencies calculated for IDH are shown in Table 2.3. For IDH-I locus all the five regions showed similar allelic frequencies, these being cent percent **S** allele (**S**=1.00). The allele frequencies at IDH-II locus were similar in Cochin, Mangalore and Madras populations with cent percent **F** allele (**F**=1.00). However. another allele **S** was also present in the population from Calicut region at a lower frequency than that of **F** allele. The frequencies of **S** and **F** allele were 0.09 and 0.91 respectively. On the other hand frequency of **S** allele was higher (**S**=0.63) than that of the **F** allele (**F**=0.37) in Mandapam population. Thus a comparison of allele frequencies of IDH-II locus in Calicut and Mandapam

populations showed significantly different values suggesting that these two populations may be genetically different. Though allelic frequencies of IDH-II locus are identical at Cochin, Mangalore and Madras, their geographic positions with reference to genetically different Calicut and Mandapam populations suggest that they are also genetically isolated populations as the each of the former two populations appeared to be a barrier between any other two populations. In other words, comparison of IDH second locus reveals that Cochin is non-polymorphic while Calicut is polymorphic; Calicut is polymorphic while Mangalore is non-polymorphic; Mandapam is polymorphic while Madras is non-polymorphic, viz. polymorphic Calicut population acts as a barrier between monomorphic Cochin and Mangalore populations.

Table 3 shows the values of the observed and expected genotype distributions at IDH-II locus in the sample populations. In Calicut population, the observed values are in agreement with the Hardy-Weinberg proportions, but significant deviations between the observed and expected values existed in Mandapam population, mainly due to excess of **F** homozygotes (TBL.3).

4.1.7 LACTATE DEHYDROGENASE (LDH)

Zymogram patterns of lactate dehydrogenase (LDH) in Sardinella longiceps from Cochin, Calicut, Mangalore, Mandapam and Madras are shown in Figure 16,17 and Plate 16,17. The zymogram showed three zones of activity which were attributed to three loci. They were designated as LDH-I, LDH-II and LDH-III indicating zones of increasing electrophoretic mobility. In all the five populations sampled LDH-I (0-1 mm) having a single band at the original sample application position was found to be non-poly-

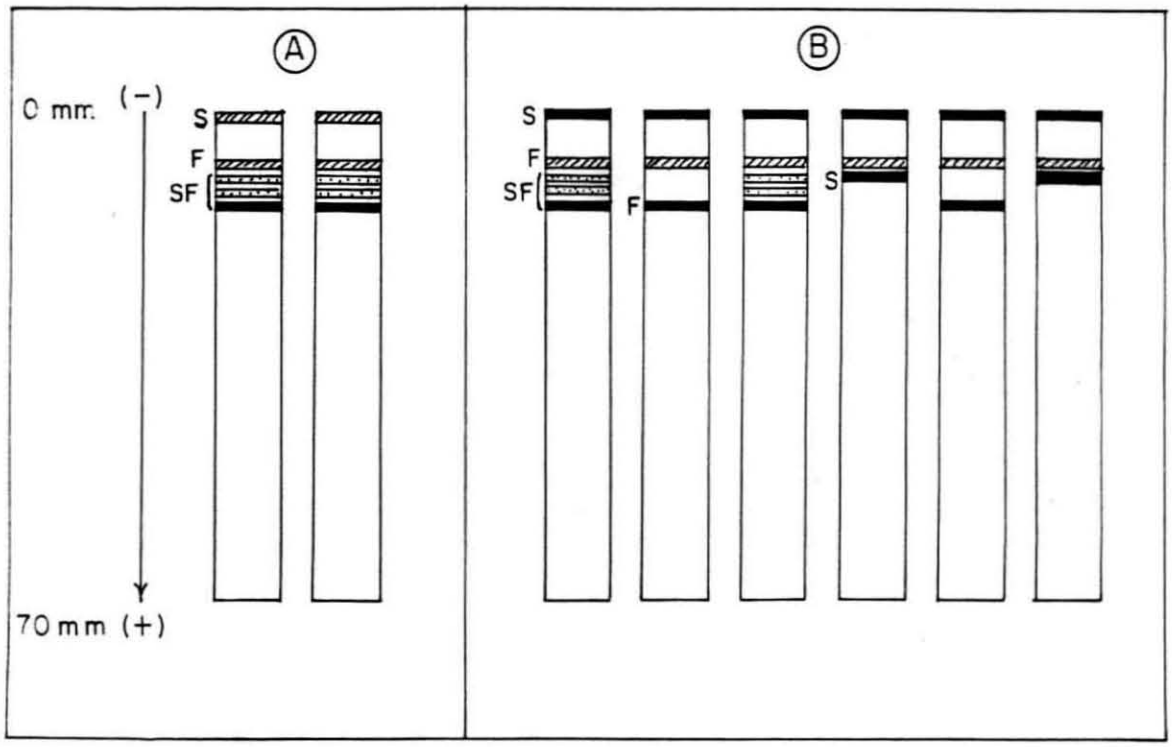
FIGURE 16 AND PLATE 16

Zymogram patterns of Lactate Dehydrogenase in

S. longiceps populations from

		Locus		
A -	Cochin	I	= 0-1	mm
B -	Calicut	II	= 4-8	mm
C -	Mangalore	III	= 9-14	mm

FIG. 16



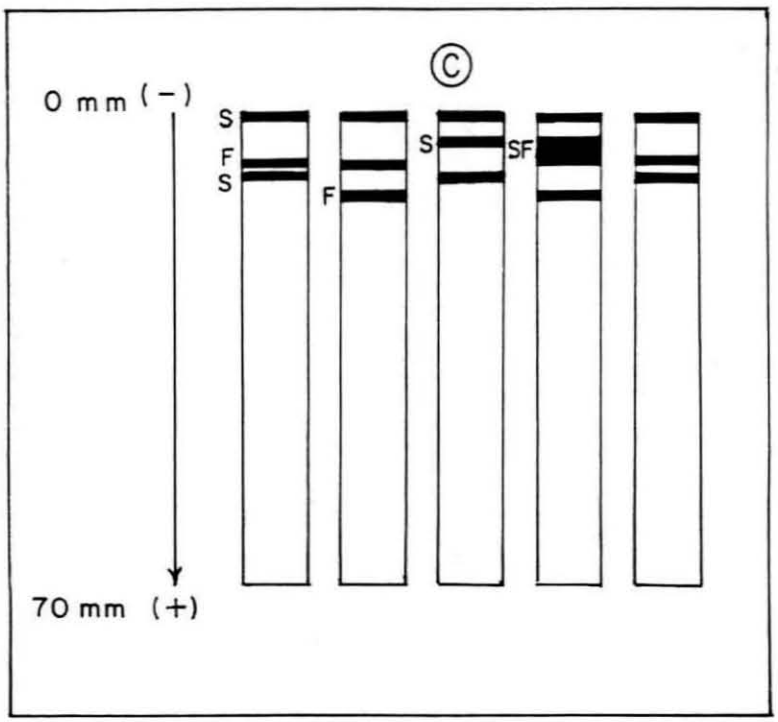


PLATE 16

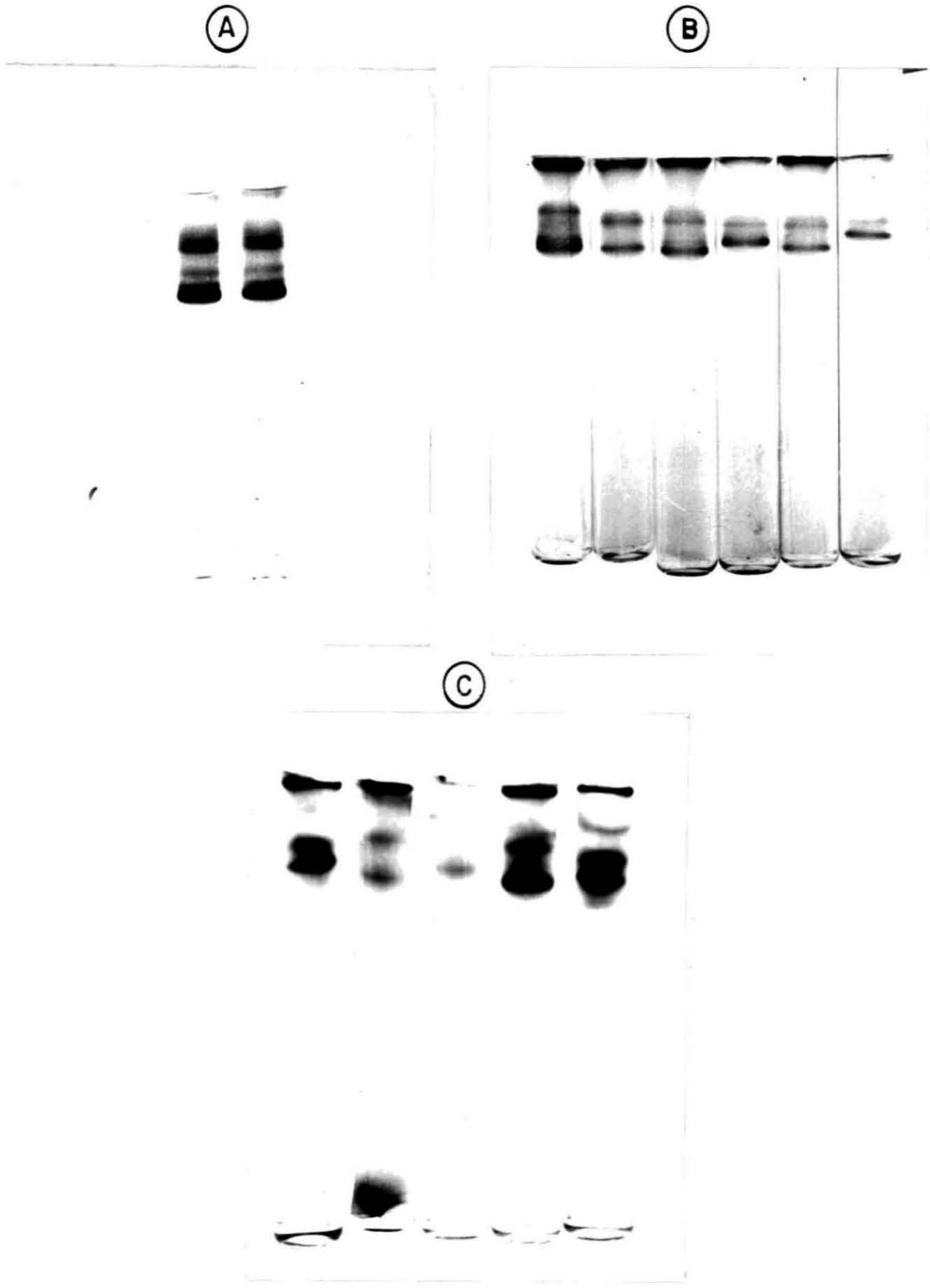


FIGURE 17 AND PLATE 17

Zymogram patterns of Lactate Dehydrogenase

in S. longiceps populations from

D - Mandapam

E - Madras

Locus

I = 0-1 mm

II = 4-8 mm

III = 9-14 mm

FIG. 17

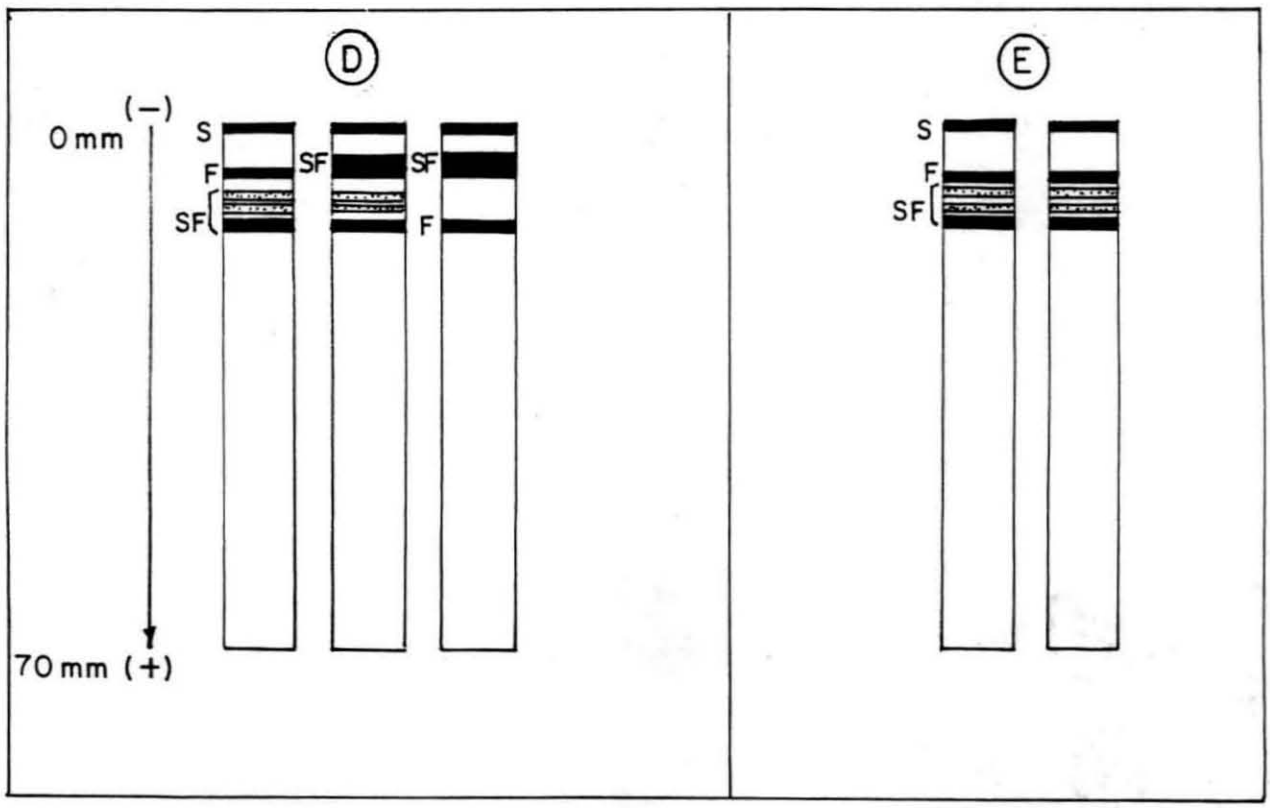
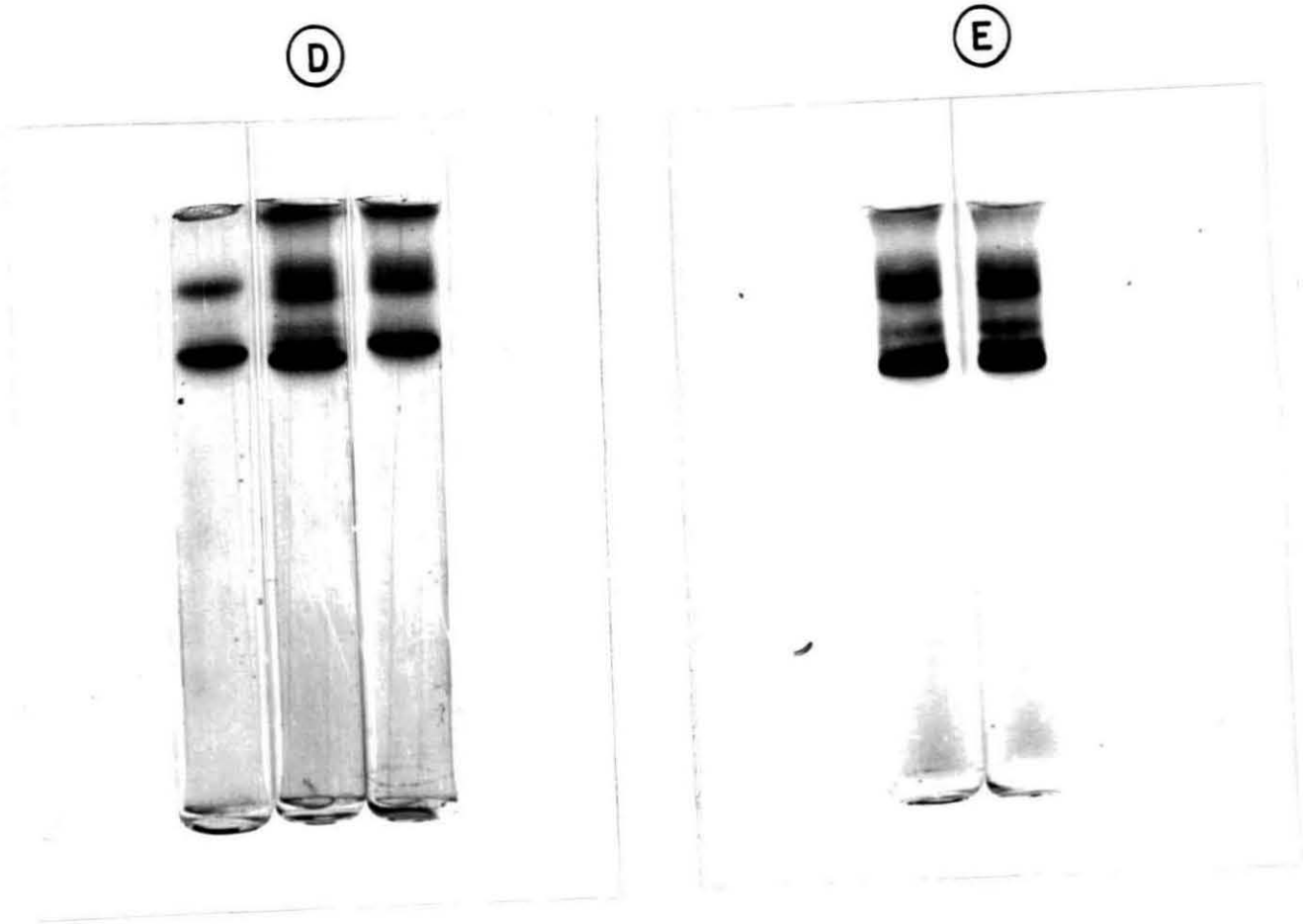


PLATE 17



morphic. LDH-II was polymorphic in both Mangalore and Mandapam populations with fast band **F** (7.0 mm) as predominant allele, whereas it was non-polymorphic in Cochin, Calicut and Madras populations; where only a single fast band **F** (7.0 mm) was observed. LDH-III appeared to be polymorphic in all the five populations. All the specimens from Cochin and Madras showed only five banded heterozygote patterns whereas both homozygotes and heterozygotes were present in samples from Calicut, Mangalore and Mandapam regions. Only three of the five bands of the heterozygotes are clearly visible in the plate whereas the other two bands were too light to be seen in the photograph, the reason of which will be discussed elsewhere (PLT.16,17). The zymogram patterns of LDH at LDH-II and III loci in a few specimens from Mangalore appeared to be different from that of all other regions. As visible in the photograph (PLT.16C), in 1st and 5th tube the two thick dark bands at the region of II and III loci are seen as very closely moved. Such a pattern is expected to occur when fast homozygote band of II locus and slow homozygote band of III locus are present together in the same individual. Such individuals were also present in Calicut as shown in the photograph (PLT.16B), particularly in the 6th tube where the bands being narrow and light, they appeared a little less closer than that of Mangalore region.

The allelic frequencies calculated for different loci are shown in Table 2. The allelic frequencies at LDH-II locus were similar in Cochin, Calicut and Madras populations, these being cent percent **F** allele in these stations. However, another allele **S** was also present in the populations from Mangalore

and Mandapam at lower frequencies than that of **F** allele which was again the predominant allele. Thus a comparison of these allelic frequencies at LDH-II locus indicate that their values are significantly different between Cochin/Calicut (**F**=1.00) and Mangalore/Mandapam (**F**=0.84//0.66) and Madras (**F**=1.00) (TBL.2). The frequencies of **S** and **F** alleles at LDH-III locus are closely comparable between the populations. The reason for the occurance of equal frequencies of 0.5 each for **S** and **F** allele in the populations from Cochin and Madras is due to the presence of cent percent heterozygotes in these two regions.

Table 3 shows the values of the observed and expected genotype distributions in the sample populations. The chi-square values are highly significant at Cochin, Mangalore and Madras (TBL.9) due to excess of heterozygotes particularly at LDH-III locus, whereas it was due to excess of homozygotes at LDH-II locus in Mangalore.

4.1.8 MALATE DEHYDROGENASE (MDH)

MDH zymogram patterns in Sardinella longiceps sampled from different areas like Cochin, Calicut, Mangalore, Mandapam and Madras are shown in Figure 18 and Plate 19. The zymogram patterns of all the five regions examined showed MDH enzyme activity in two different gel positions, the first at the 0-1 mm to 5 mm designated as MDH-I locus and the second at 9 mm designated as MDH-II locus. MDH-I locus was found to be monomorphic in Cochin, Calicut and Mandapam populations where only the fast phenotype (**F** band) was expressed compared to the polymorphic pattern of

FIGURE 18 AND PLATE 18

Zymogram patterns of Malate Dehydrogenase

in S. longiceps populations from

		Locus		
A -	Cochin/Calicut/ Mandapam	I	= 0-5	mm
B -	Mangalore/Madras	II	= 8-9	mm

FIG. 18

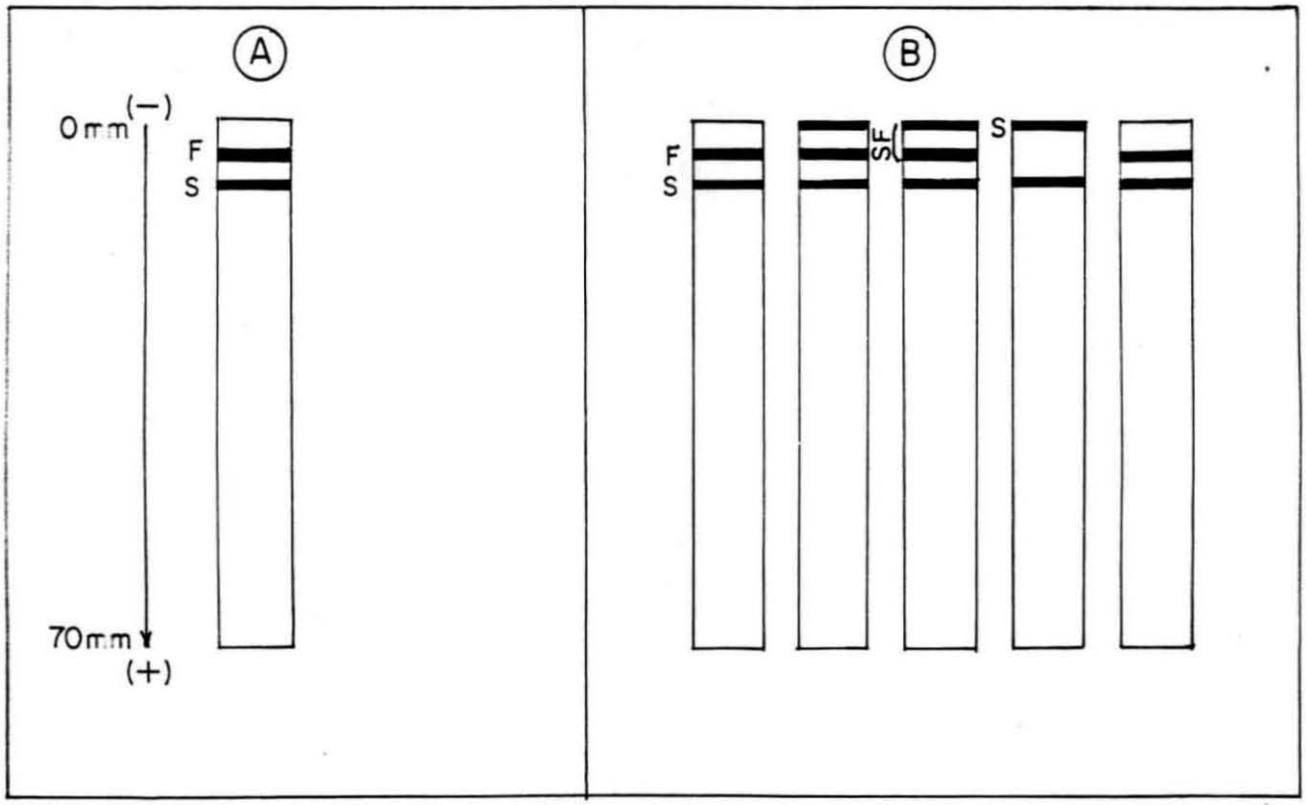
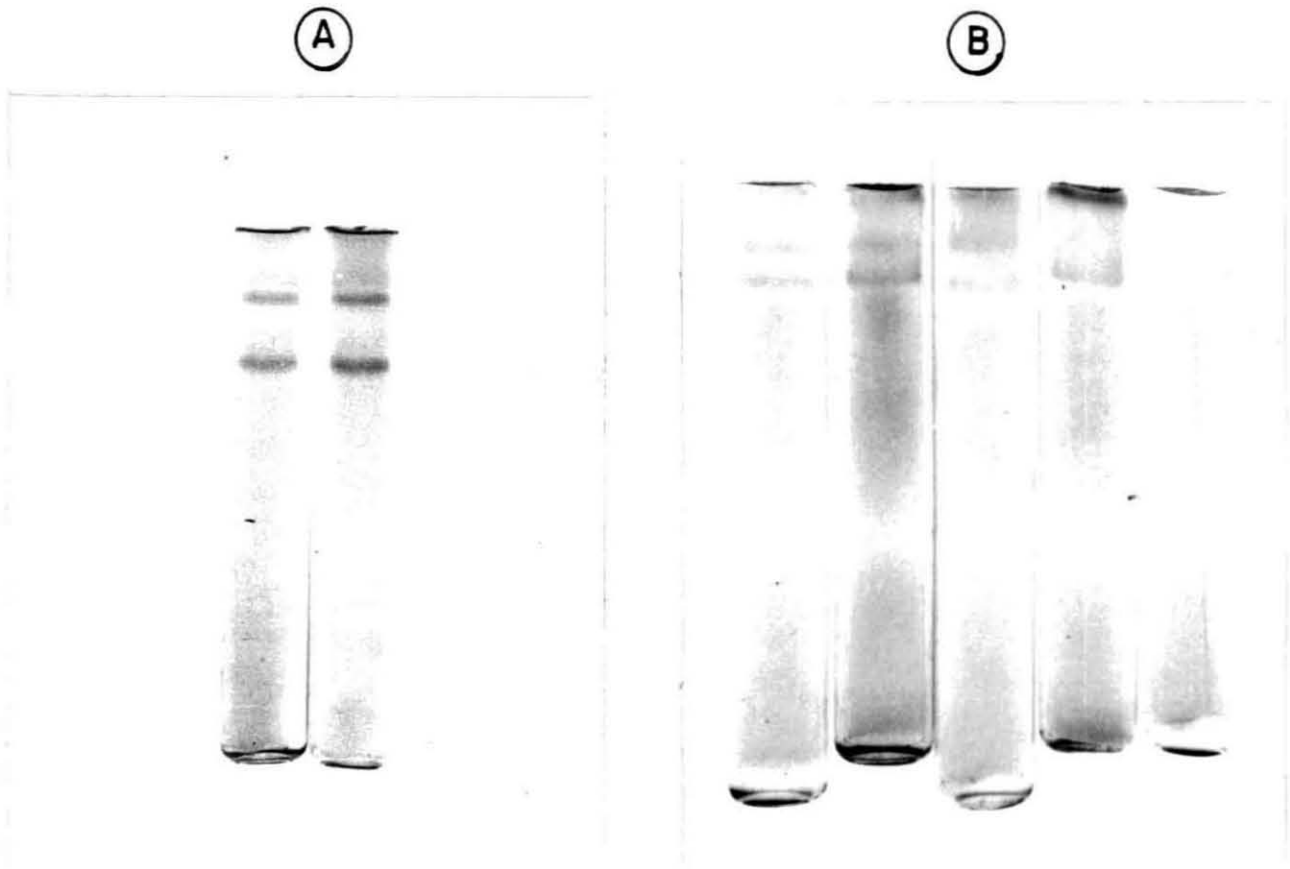


PLATE 18



Mangalore and Madras. The variant phenotypes observed in these two populations were slow moving **S**, fast moving **F** and their combinations **SF** (FIG.18A and B, PLT.18A and B). The second locus showed monomorphism in all the stations examined with only one band (**S** band). Thus the MDH enzyme patterns in Cochin, Calicut and Mandapam populations appeared to be just two banded (FIG.18A, PLT.18A). On close comparison with that of polymorphic patterns of Mangalore and Madras, the first band was in equal position of **F** band of polymorphic MDH-I locus, whereas the second band at monomorphic second locus was identical in all the five populations (FIG.18B, PLT.18B).

Allelic frequencies calculated for MDH-I and II locus are summarized in the Table 2. Mangalore and Madras populations alone showed polymorphism at MDH-I locus. The frequency for the major allele **F** was 0.57 and 0.70 in Mangalore and Madras populations respectively. Their allelic frequency differences as well as distant geographical positions separated by other distinct populations indicate that these two populations are genetically different. The non-polymorphic MDH-I locus in Mandapam and its polymorphic nature in Madras clearly suggests their genetic difference viz. **F** allele appears to be fixed in Mandapam, while it is only predominant in Madras population. In this respect monomorphic Cochin and Calicut populations are genetically distinct from polymorphic Mangalore population.

The observed and expected values of genotype frequencies are shown in the Table 3. The expected frequencies are in close agreement with the observed frequencies. Therefore, the chi-square values are with in the limits,

these being 1.96 (P>0.05) and 0.726 (P>0.05) for Mangalore and Madras populations respectively (TBL.9). Thus the observed MDH-I locus polymorphism in S. longiceps is balanced in nature.

4.1.9 XANTHINE DEHYDROGENASE (XDH)

Figure 19 and Plate 19 shows zymogram patterns of xanthine dehydrogenase (XDH) in Sardinella longiceps tested from Cochin, Calicut, Mangalore, Mandapam and Madras. From different zymogram patterns, it was inferred that XDH is controlled by two presumtive loci. The activity of XDH-I locus occured between 0-7 mm gel position whereas XDH-II locus activity was observed at 10-11 mm position. Only XDH-I locus showed phenotype variation (FIG.19, PLT.19). The polymorphism at XDH-I locus produced fast band (**F** homozygote) and slow-fast bands (**SF** heterozygote). However, slow homozygote (**S** band) was not observed in any of the population. In Mangalore and Madras populations (PLT.19B and D) the mobility of XDH-I fast band was slightly more than that of Cochin, Calicut and Mandapam (PLT.19A and C). Interestingly the mobility of XDH-II slow band seemed to be more slower in Mangalore and Mandapam populations. Similar phenomenon was also observed, in GDH enzyme as reported earlier.

The allelic frequencies of XDH first and second loci are summarized in Table 2. The polymorphic XDH-I locus showed significant allele frequency differences between regions except Mangalore and Mandapam. The predominant allele was **F** in all the populations, the frequency of which ranged from 0.54 to 0.92. The Mangalore and Mandapam populations showed same allele frequency of 0.71 each for **F** allele and 0.29 for **S** allele respectively. Significant

FIGURE 19 AND PLATE 19

Zymogram patterns of Xanthine Dehydrogenase in

S. longiceps populations from

		Locus	
A -	Cochin/Calicut	I	= 0-7 mm
B -	Mangalore	II	= 10-11 mm
C -	Mandapam		
D -	Madras		

FIG. 19

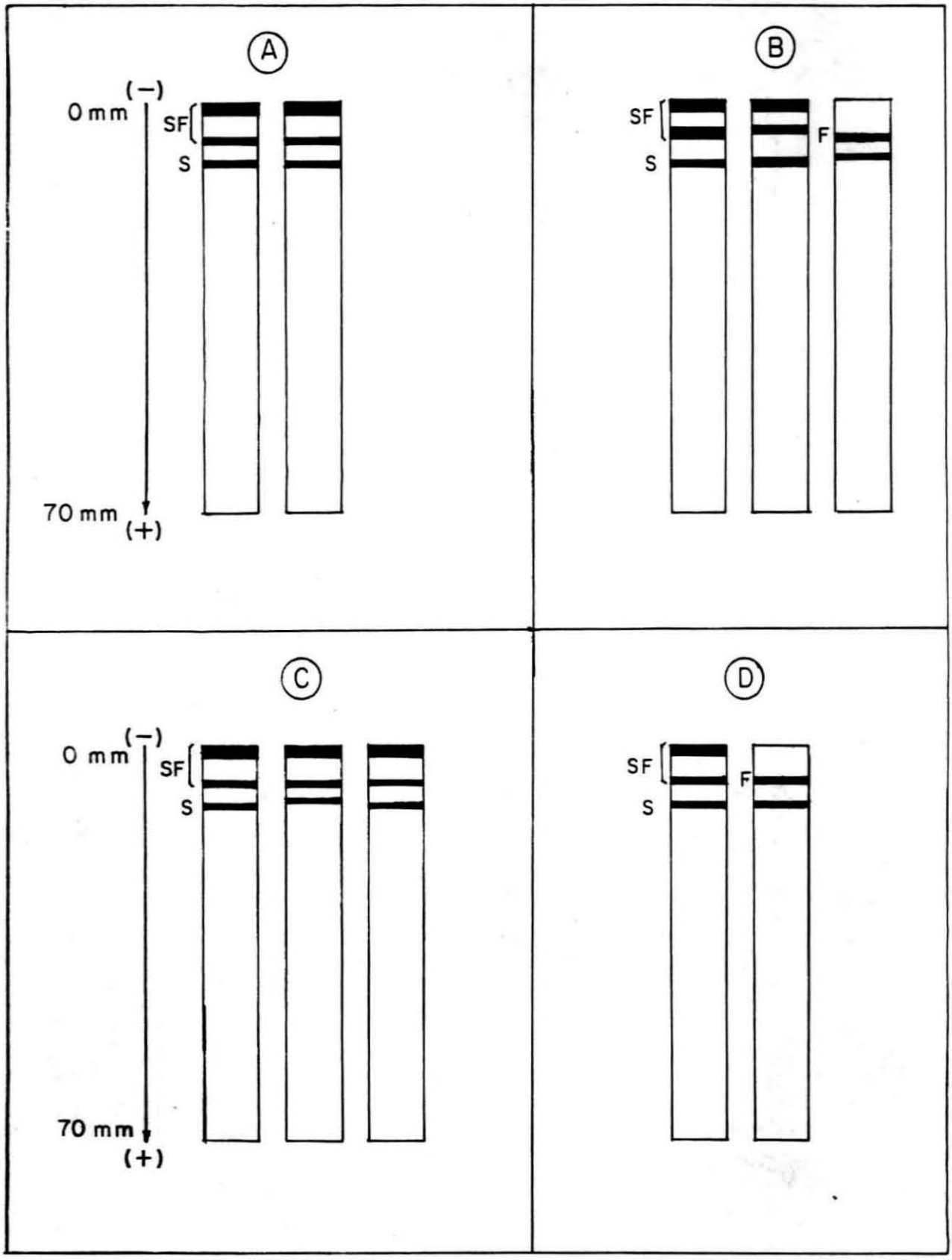
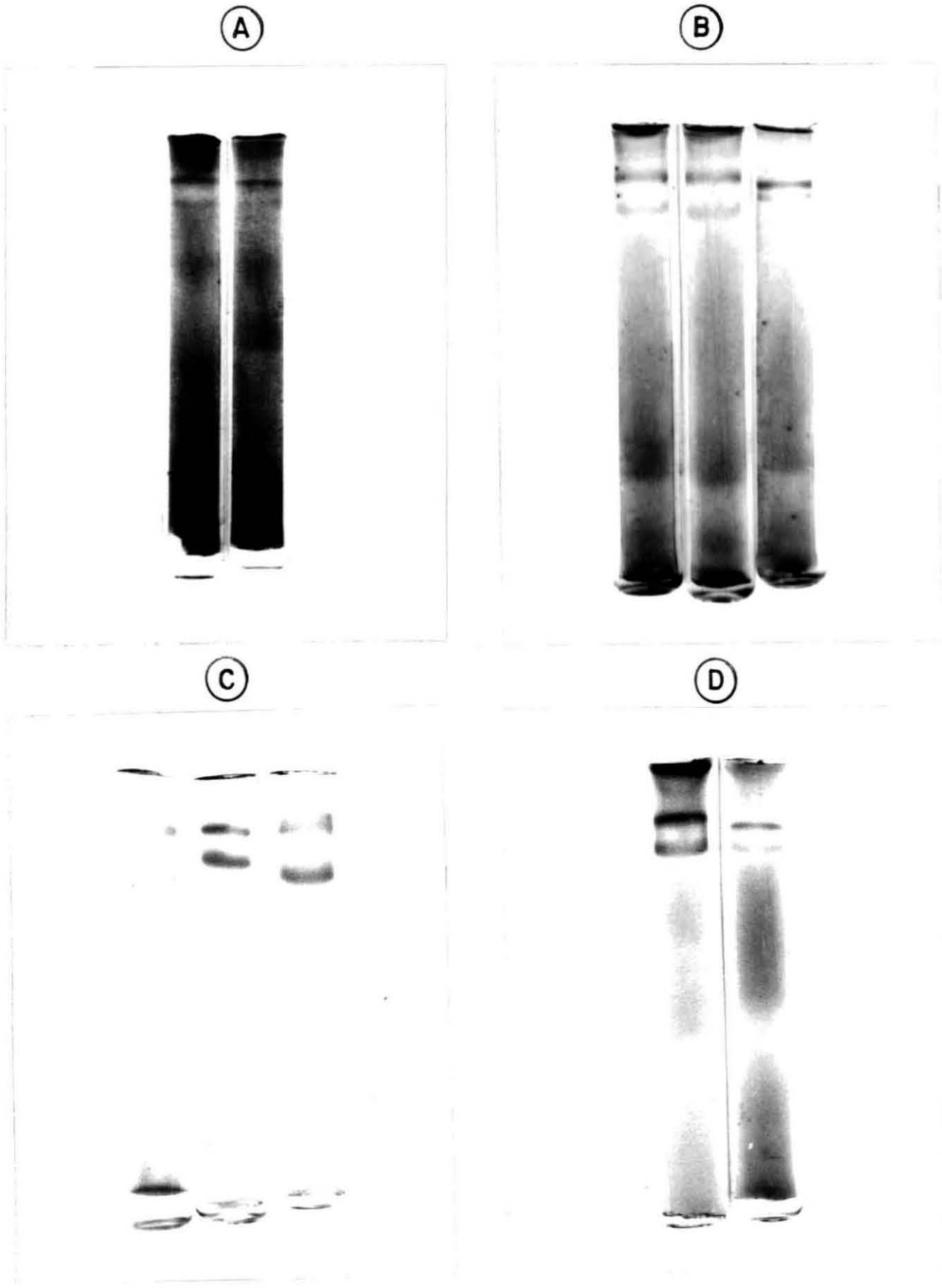


PLATE 19



allele frequency differences between regions suggest genetic heterogeneity of different populations.

Values of observed and expected genotype frequencies in the sample populations are tabulated in the Table 3 . Significant heterozygote excess was observed at Calicut population whereas only considerable deviation from Hardy-Weinberg equilibrium was observed in Mangalore, Mandapam and Madras due to excess of heterozygotes. However, Cochin population showed good agreement with equilibrium conditions.

4.2 ANALYSIS OF DATA

4.2.1 Average Polymorphism

Details on the total number of polymorphic loci, average polymorphism in different populations of S. longiceps and the average polymorphism in the species are presented in Table 7. A total of 25 loci belonging to nine enzyme systems were considered for calculation of all the 5 populations. Average number of polymorphic loci ranged from 0.33 in Cochin to 0.55 in Mandapam, giving an average 0.41 in S. longiceps. Thus these results revealed considerable differences in average polymorphism between the populations.

4.2.2 Average number of alleles per locus

Table 8 shows comparison of average number of alleles at 25 loci in S. longiceps from 5 different populations. Total alleles ranged from 33 in Cochin to 37 in Mandapam. The average number of alleles in each population ranged from 1.28 in Cochin to 1.49 in Mandapam. The average number

of alleles in Sardinella longiceps was 1.37. The data on the average number of alleles per locus also showed considerable population differences as revealed by average polymorphism.

4.2.3 Heterozygosity

The proportions of heterozygotes at each of the 25 loci for all the 5 populations sampled are shown in Table 4. The average heterozygosity for 9 different enzymes of the 5 populations of S. longiceps are given in Table 5. Average heterozygosity in the five populations ranged from 0.16 in Mangalore to 0.24 in Mandapam and it was 0.21 in S. longiceps species. The enzyme wise average heterozygosity varied from 0.06 in G6PD to 0.37 in EST. These results thus revealed significant heterozygosity differences among the five populations of S. longiceps. In other words, very high genetic variability existed within the species.

4.2.4 Genetic Identity and Distance

Table 10 shows computed values of genetic identity and corresponding distance for five different populations of S. longiceps. A total of 250 comparisons involving 25 enzyme loci and five populations were made for obtaining the average G.I and G.D. The estimated mean values of genetic identity and their corresponding genetic distance ranged from 0.846 (Mangalore/Mandapam) to 0.967 (I) (Cochin/Mangalore) and 0.033 (Cochin/Mangalore) to 0.166 (D) (Mangalore/Mandapam) respectively. Thus the lowest genetic identity (0.846) or the corresponding highest genetic distance (0.166) was found between Mangalore and Mandapam followed by Calicut and Mandapam,

suggesting that east west populations are more genetically different than between populations of each coast. The lowest genetic distance was found between Cochin and Mangalore, closely followed by Cochin and Calicut. Interestingly, as expected, genetic similarity is more among populations of west coast than between west and east coast populations. Because, average genetic identity values of west and east coast populations were 0.95 and 0.90 respectively. Correspondingly average genetic distance among three west coast populations is less than that between west coast and east coast populations, these being 0.0685 and 0.1165 respectively for the two groups. Another interesting finding is that average genetic distance is higher between Mandapam/Madras (0.0856) than that of Cochin/Calicut/Mangalore (0.0685), suggesting Mandapam and Madras populations are more dissimilar and distant than between populations of west coast.

5. DISCUSSION

The traditional concept on unit stock in fisheries research and management as described by Marr (1957) and Muzinic and Marr (1960) could not contain the micro and macro evolutionary processes known to occur at gene levels of species organisation (Ayala and Kiger, 1980). Hence, the significance of application of genetic principles in fisheries research and management was well recognised at the ICES special meeting (deLigny, 1971). Recently THE STOCK CONCEPT INTERNATIONAL SYMPOSIUM (Anon, 1981) again reiterated and elaborately discussed various aspects of stock concepts in fisheries research and management. The importance of application of gene controlled proteins/enzymes in detecting and identifying reproductively isolated genetic stocks of fish resources was strongly emphasised in the stock symposium (Altukhov, 1981; Allendorf and Phelps, 1981; Ihssen et al., 1981a; Mac Lean and Evans, 1981).

Detection and identification of specific proteins/enzymes were made easier by gel electrophoresis and zymogram methods developed by Smithies (1955) and Hunter and Markert (1957) respectively. The combined application of these two biochemical techniques has revealed good amount of biochemical genetic polymorphism in almost all the fish and shell fish species tested throughout the world. An overall analysis of the averages of polymorphic loci, number of alleles per locus, heterozygosity etc. would reveal the degree of genetic variability between the populations belonging to the species.

A comparison of the nature of polymorphism between two or more populations has enabled the investigators to establish genetic homogeneity

or heterogeneity of the populations of a particular species. The degree of homogeneity or heterogeneity is measured primarily by the allelic frequencies at several enzyme loci examined. Significant differences at many gene loci are considered as evidence for the stock differences whereas non significant allelic differences indicate genetic homogeneity of the populations (Jamieson, 1974; Ayala and Kiger, 1980; Allendorf and Phelps, 1981; Altukhov, 1981). Further, comparative analysis of genetic identity and genetic distance according to the method of Nei (1972) can also throw some light on the present genetic relationship attained by different populations in the process of unknown evolution (Ayala and Kiger, 1980; Utter, 1987). The identification of genetic unit stock structure of a fish species that is being exploited as a single stock fishery is the first step in preventing the collapse of the fishery itself. For example, the collapse of the fishery of Pacific sardine, Sardinops sagax was thought to be the consequence of gradual break down of its suspected subpopulations (Radovich, 1982, Hedgecock, 1986). The Indian oil sardine Sardinella longiceps is heavily exploited as a single stock fishery. Besides, significant fluctuation in its total landings has become an undesirable nature of the Indian oil sardine fishery (Antony Raja, 1973). Hence the possible role of composite population structure of S. longiceps in its short and long term fluctuations cannot be ruled out.

In the above background, the main objective of the present investigation was to study biochemical genetic composition of populations of Sardinella longiceps with a view to identify discrete genetic stocks of the species, if any. To achieve the above objective, standardized biochemical genetic

techniques were applied at all levels of the investigation (TBL.1). As a result essentially required data in the form of zymograms (FIGS.2-19), allelie frequencies (TBL.2), proportion of polymorphic loci (TBL.7), average heterozygosity (TBL.5), number of alleles per locus (TBL.8), average values of genetic identity and distance (TBL.10) at twenty five gene loci belonging to nine enzyme systems were collected for five populations of S. longiceps. Each of these enzyme systems was analysed separately to obtain the present genetic nature of each population. These results have been discussed objectively as well as in the light of comparable and relevant results reported by others in the same and other species of fishes. For making the discussion natural, important genetic aspects of each populations of S. longiceps have been presented enzyme wise. Though a total of about 50 enzyme systems were identified in different organisms by gel electrophoresis and zymogram techniques (Shaw and Prasad, 1970) only nine enzyme systems could be analysed in the present investigation. There are two main reasons for selecting these specific nine enzyme systems. The first was that these enzymes were already known to be actively present and polymorphic in many fish species. The second reason is, it was not practical to include all the enzyme systems within the limited time, budget and facilities available for the investigation.

Quantitative and qualitative deviation of certain genetic characteristics from that of ideal have also been discussed separately under the heading general discussion. This coherent form of discussions enabled to get an insight into the population genetic structure of S. longiceps and to make the conclusion scientifically more rewarding.

5.1 ALCOHOL DEHYDROGENASE (ADH)

Zymogram patterns of liver ADH in Sardinella longiceps showed intra-species genetic polymorphisms in Calicut and Mandapam populations while monomorphism in Cochin, Mangalore and Madras populations. Thus these two different patterns of ADH indicated genetic differences between these two groups of populations. Similar genetic differences were also observed at IDH locus discussed elsewhere. ADH has been reported as monomorphic in Artic charr (Ryman and Stahl, 1981), brown trout (Skaala and Jorstad, 1987) and Alaskan chinook salmon (Gharrett et al., 1987). However, ADH has been reported as polymorphic in Oryzias (Sakaizumi et al., 1983), cutthroat trout (Campton and Utter, 1987) and rainbow trout (Berg and Gall, 1988). Buth (1980) reported both monomorphic and polymorphic ADH in different populations of Hypentelium nigricans which phenomenon is comparable to the present findings in S. longiceps.

An overall comparison of allele frequencies in these two groups of populations naturally indicated significant frequency differences. For example, slow **S** allele frequency was cent percent in Cochin, Mangalore and Madras populations, whereas it varied between 0.72 to 0.95 in Calicut and Mandapam populations reinforcing the above stated genetic differences (TBL.2). The ADH allele frequency patterns also indicated significant differences between closely related regions like Cochin and Calicut, Calicut and Mangalore and Mandapam and Madras. Though allele frequencies at ADH-I and II are identical in populations from Cochin, Mangalore and Madras, they are to be considered as genetically isolated populations due to the presence of polymorphic Calicut

population between Cochin and Mangalore and polymorphic Mandapam population between Mangalore and Madras. That is, a polymorphic population acts as a barrier between two non-polymorphic populations. Though allele frequencies at ADH-I locus was similar between Calicut and Mandapam, allele frequency of second locus indicated considerable differences suggesting their possible genetic differences, it being 0.95 in Calicut and 0.75 in Mandapam for **S** allele. Similar allele frequency differences have been reported by various authors. Buth (1980) reported ADH polymorphism and significant allele frequency differences in his study in selected populations of Hypentelium. Present (1987) has reported highly significant allele frequency differences between different populations of Hypoblennius jenkinsi. The frequency of a and b allele from 2 populations showed an average of 0.56 and 0.44 whereas another population showed 0.20 and 0.80 for a and b allele respectively. Hardy-Weinberg equilibrium tests indicated good agreement between observed and expected values (TBL.3). The second locus at Calicut population showed perfectly balanced polymorphism.

It is really interesting to observe that populations of close proximity like Cochin and Calicut have significant allelic frequency differences, while distant populations like Cochin, Mangalore and Madras have similar allelic frequencies. Such interesting phenomenon was reported in rainbow trout (Berg and Gall, 1988). Similar phenomenon was also reported in the morphometrics of Nemipterus japonicus. Its populations from close by Kakinada and Visakhapatnam were significantly different while its population from distant Madras was not found more heterogenous than that of Kakinada and

Visakhapatnam (Rao and Rao, 1983). The authors attributed low salinity, ecological variation of Kakinada region as the possible basis for the observed population differences. However, in the present case no such ecological differences have been reported existing in Cochin and Calicut. Above all, the present observed differences are based on protein allelic differences, not influenced by non-genetic factors like salinity. Besides, such apparent genetic similarity over long distances is characteristic of many marine teleost species such as Katsuwonus pelamis (Richardson, 1983), Stegastes fasciolatus (Shaklee, 1984), Clupea spp. (Grant, 1984; Grant and Utter, 1984; Ryman et al., 1984), Gadus morhua (Mork et al., 1985) and Hoplostethus atlanticus (Smith, 1986) as reported by Milton and Shaklee (1987).

5.2 ALDEHYDE OXIDASE (AO)

Aldehyde oxidase was also found highly polymorphic in Sardinella longiceps tested from Cochin, Calicut, Mangalore, Mandapam and Madras. Though first locus of this enzyme showed monomorphism in all the populations tested, the second and third locus showed polymorphism. The range of **S** allelic frequencies, 0.31 in Cochin and 0.73 in Mandapam indicated genetic heterogeneity among the populations. However, the reason for getting equal frequency of 0.50 each for slow and fast allele at Calicut and Mangalore stations is the observance of cent percent heterozygotes in these two populations (TBL.2).

The third locus of aldehyde oxidase was polymorphic only in Cochin population, while all the other populations showed monomorphism. Thus Cochin population appeared to be genetically very different from all other

populations. Cochin population showed **S** allele frequency as 0.19 whereas **F** allele showed dominancy (F=0.81). Though Calicut, Mangalore, Mandapam and Madras showed monomorphism, it is very interesting to discuss their gene frequencies. That is, while the Calicut and Mangalore populations showed cent percent **F** allele, Mandapam and Madras populations both on east coast showed cent percent **S** allele. Therefore, the **S** allele fixation at Mandapam and Madras and **F** allele fixation at Calicut and Mangalore clearly indicates strong stock differences between Calicut/Mangalore group and Mandapam/ Madras group of populations. Thus, an overall comparison of second and third loci suggests significant genetic differences among all five populations except between Calicut and Mangalore.

Though available reports on biochemical genetic studies in other fishes show that aldehyde oxidase was not investigated as a potential genetic marker, it has been studied by many investigators in fishes; for example, Atlantic halibut (Fevolden and Haug, 1988) and shell fishes (Berglund and Lagercrantz, 1983; De Matthaeis et al., 1983) and in cat fishes (Suzuki and Phan, 1990). However, it has been studied in detail in Drosophila (Ayala et al., 1974). Monomorphism has been reported in gummy shark (Mac Donald, 1988) and in many marine crustaceans (Lester, 1979, 1983; Fuller and Lester, 1980; Redfield et al., 1980) as reported by Philip Samuel (1987).

Polymorphic aldehyde oxidase has been reported in P. indicus by Philip Samuel (1987) showing two alleles with two banded heterozygote. It was also found highly polymorphic with two banded heterozygote in Mugil cephalus (Vijayakumar, 1992) as also observed in S. longiceps here. Though gene fre-

quencies were same in both Cochin and Madras for aldehyde oxide enzyme in Mugil cephalus, considerable gene frequency difference was observed in Orissa population and thus indicating genetic heterogeneity of Orissa population. De Matthaeis et al. (1983) have reported both polymorphism and monomorphism in their study to find out genetic differentiation between Penaeus kerathurus and P. japonicus. It is interesting to note that A0-1 showed monomorphism in populations of both P. japonicus and P. kerathurus, a comparable pattern to that of the present investigation. It is also interesting to note that A0-2 was polymorphic in P. japonicus as was also noticed here in S. longiceps. Further the observation of a third locus and its genetic differences in S. longiceps appears to be a first report of its kind.

Thus the reports of the genetic nature of aldehyde oxidase enzyme in different species as well as between populations of a particular species suggest its important role at different levels of species organisation. These reports also suggest its suitability as a potential genetic marker for differentiation of species as well as populations.

A comparison of Chi-squared values at locus II of aldehyde oxidase indicated significance due to excess of heterozygotes in Cochin, Calicut and Mangalore whereas its significance due to excess of homozygotes was present in Madras population. The highly significant deviation in Chi-squared values noticed in Calicut and Mangalore was due to cent percent heterozygotes in these two regions. The Chi-squared values at AO-II at Mandapam and AO-III at Cochin were non significant (TBL.9).

Table: 9

Chi-squared values of the genotype frequencies at 25 loci with their levels of significance in Sardinella longiceps

Sl. No.	Locus	Populations				
		CHN	CCT	MRE	MDM	MAD
1.	ADH I	-	2.22	-	3.08	-
2.	ADH II	-	0.055	-	2.22	-
3.	AO I	-	-	-	-	-
4.	AO II	9.92 (+) P>0.001	24 (+) P < 0.001	24 (+) P< 0.001	3.03	6.13 (+) P> 0.01
5.	AO III	2.57	-	-	-	-
6.	EST I	3.83	3.09	0.118	-	-
7.	EST II	16.0 (+) P<0.001	0.961	0.179	0.036	10.8 (+) P> 0.001
8.	EST III	8.63 (+) P> 0.001	0.115	5.69 (+) P> 0.01	-	-
9.	EST IV	3.19	0.042	0.316	5.52 (+) P> 0.01	0.622
10.	G6PD I	-	-	-	-	-
11.	G6PD II	-	-	-	-	-
12.	G6PD III	-	-	-	1.61	20.41 (+) P <0.001
13.	G6PD IV	-	-	-	-	-
14.	G6PD V	-	-	-	-	-
15.	GDH I	7.91 (+) P> 0.001	2.28	17.18 (+) P < 0.001	0.668	0.247
16.	GDH II	-	-	-	19.05 (+) P < 0.001	-
17.	IDH I	-	-	-	-	-
18.	IDH II	-	0.64	-	9.71 (+) P > 0.001	-
19.	LDH I	-	-	-	-	-
20.	LDH II	-	-	15.16 (+) P < 0.001	5.14 (+) P > 0.01	-

Table: 9 contd...

Sl. No.	Locus	Populations				
		CHN	CCT	MRE	MDM	MAD
21.	LDH III	42 (+)	0.0015	21.91 (+)	4.04 (+)	24 (+)
		P < 0.001		P < 0.001	P> 0.01	P < 0.001
22.	MDH I	-	-	1.96	-	0.726
23.	MDH II	-	-	-	-	-
24.	XDH I	0.43	16.27 (+)	4.07 (+)	4.07 (+)	4.04 (+)
			P <0.001	P > 0.01	P> 0.01	P > 0.01
25.	XDH II	-	-	-	-	-

+ indicates the significant chi-squared value

5.3 ESTERASE (EST)

Esterase enzyme system of Sardinella longiceps in muscle tissue showed genetic polymorphism at all four loci detected. Out of the four loci, first locus showed polymorphism in populations of west coast and monomorphism in populations of east coast, suggesting significant east west regional stock differences. The frequency of major allele **S** was from a range of 0.73 to 0.93 in Cochin, Calicut and Mangalore and cent percent in Mandapam and Madras. Though **S** allele is predominant in Cochin, Calicut and Mangalore, considerable frequency difference was shown by Mangalore population (TBL.2). The second, third and fourth loci were highly polymorphic in all the four populations. The allele frequencies at the second locus in Cochin, Calicut and Mangalore were considerably different among themselves, whereas these were again almost similar between Mandapam and Madras (TBL.2). Though predominant allele in Cochin, Mangalore, Mandapam and Madras was **S** allele, the Calicut population differentiated itself by having **F** as its predominant allele. The allele frequencies at third locus were considerably different in all the populations except between Calicut and Mangalore. Besides, the predominant allele between populations appears to be considerably different at third locus except between Calicut and Mangalore (TBL.2). On the other hand, the nature of allele frequencies at fourth locus indicated considerable difference in Mangalore population in the west coast and also between Mandapam and Madras in east coast. Thus, an overall comparison of allele frequencies at each of four esterase locus between populations and between east and west coast suggests genetic stock differences in S. longiceps tested

in the present investigation. However, Mandapam and Madras populations could be clearly differentiated only by third locus, where predominant alleles were different.

There are many published reports on the application of intraspecies esterase polymorphism for the identification of stock differences. The presence of polymorphic as well as non-polymorphic esterase locus reported in many other fishes and crustaceans are comparable with the present findings. For example, Crozier (1987) observed three loci of which Est-3 was polymorphic. In his study out of the total five populations of Angler fish, three showed monomorphic 100 allele whereas other two populations showed polymorphism. Similarly, Shaklee and Samollow (1984) detected three esterase loci out of which Est-3 was polymorphic. Andersson et al. (1983) identified three esterase loci in Arctic charr, where one locus was found to be monomorphic and others polymorphic.

From different reports it is also evident that esterase enzyme can be applied for stock identification purposes. For example, Utter (1969) considered significant gene frequency differences calculated at esterase locus at a level of 0.603 and 0.828 between two populations of Pacific hake, Merluccius productus as genetic stock differences. Smith et al. (1978) identified distinct genetic stocks on the basis of significant esterase allele frequency differences in the populations of New Zealand snapper. Similarly, Altukhov (1981) detected genetic heterogeneity and reproductive isolation in salmon and red fish populations based on esterase gene frequency differences. Genetic stock differences in populations of Catostomus have been

detected by Buth and Crabtree (1982) based on esterase gene frequency differences. In their study cent percent slow allele was observed at Est-2 locus in Serpe Creek populations whereas all other populations showed varied allele frequencies ranging from 0.89 to 0.98. Similar genetic heterogeneity has also been reported by Grant (1984) in Atlantic herring populations based on esterase allele frequency differences. Richardson and Habib (1987) considered esterase as a genetic tag for identification of different genetic stocks. In a very recent study on Mugil cephalus, Vijayakumar (1992) reported genetic heterogeneity and also reproductive isolation among its three populations with regard to esterase allele frequency differences.

All the above discussed reports thus corroborate the present fingings of genetic polymorphism in esterase enzyme in S. longiceps and its application for stock differentiation in the species. All these reports also suggest that esterase enzyme systems in fishes can be a potential genetic marker for population genetic analysis.

Hardy-Weinberg tests conducted, showed significant deviation in only five out of twenty cases. Three cases showed excess of heterozygotes at Cochin (second and third locus) and Mandapam (fourth locus), whereas, two cases of excess of homozygotes were observed in second locus of Madras and third locus of Mangalore. All were significant at 5% level. Such occurrence of excess of homozygotes and excess of heterozygotes are common. Jamieson et al. (1971) have revealed an excess of homozygotes in nearly half of all samples tested in their study in Scomber scombrus as reported by Smith and Jamieson (1978). Smith et al. (1981a) detected significant excess

of homozygotes in Atlantic mackerel in seven out of eleven area samples collected in the North East Atlantic. Even after analysing the data with respect to sex, year class, size within the year class and haul, homozygotes showed their dominancy. Various other reports are also there showing excess of homozygotes at esterase locus (Mitton and Koehn, 1975; Winans, 1980; Andersson et al., 1983; Richardson and Habib, 1987; Seeb et al., 1987; Lavery and Shaklee, 1989). Excess of heterozygote at esterase locus have been reported by Smith et al. (1978) in their study to find out genetic differentiation in New Zealand snapper.

5.4 GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD)

All the populations of Sardinella longiceps from Cochin, Calicut, Mangalore, Mandapam and Madras showed identical monomorphism at G6PD-I, II, IV and V loci whereas its third locus showed polymorphism in Mandapam and Madras and monomorphism in Cochin, Calicut and Mangalore. Thus the third locus showed very significant differences in the allele frequency between populations of west and east coast. The third locus frequencies were also considerably different between Madras and Mandapam populations suggesting genetic heterogeneity. In all the monomorphic loci the cent percent slow allele was observed. However, Mandapam and Madras populations showed **F** as their major allele. The allele frequency of **F** allele being 0.76 in Mandapam and 0.53 in Madras (TBL.2). Therefore, monomorphism in west coast and polymorphism in east coast population at third locus indicate strong genetic stock differences between these two regional populations. Similarly,

significant difference in allele frequency between Mandapam and Madras also showed genetic heterogeneity of the two local populations in the east coast region.

G6PD has been reported as monomorphic in most of the fish and shell fish species such as walleye pollock (Grant and Utter, 1980), Atlantic mackerel (Smith and Jamieson, 1980), black seabrem (Sumantadinata and Taniguchi, 1982), Arctic charr (Andersson et al., 1983) and Penaeus japonicus (Sbordoni et al., 1986). De Matthaeis et al. (1983) in their study on genetic differentiation between Penaeus kerathurus and P. japonicus found two loci of G6PD as monomorphic in both species. Similarly, Lavery and Staples (1990) found monomorphic G6PD in Penaeus esculentus and P. semisulcatus. Interestingly, Winans (1980) observed polymorphism in only one population of Chanos chanos out of the total fourteen populations. In his study, the most common allele (100) showed 0.95 in the polymorphic population, while all the monomorphic populations showed cent percent 100 allele. However, considerable allele frequency differences were found between samples of Hoplostethus atlanticus (Smith, 1986). Out of the seven populations sampled in his study two showed monomorphism whereas other five populations showed polymorphism. The frequency of major allele ranged from 0.938 to cent percent. Thus the reports of monomorphism and polymorphism at certain loci of G6PD enzyme in other fishes and shell fishes strongly corroborate similar findings in Sardinella longiceps tested in the present investigation. However, the present findings appear to be considerably different in the degree of genetic polymorphism at G6PD loci, because the degree of poly-

morphism reported in Chanos chanos (Winans, 1980) and Hoplostethus (Smith, 1986) is very low, it being 0.957 to cent percent and 0.938 to cent percent respectively, whereas it was ranging from 0.53 to 1 for the predominant allele in S. longiceps. Another significant difference noticed in the present investigation is the predominance of **F** allele in polymorphic locus.

Hardy-Weinberg equilibrium tests conducted showed balanced polymorphism in Mandapam population whereas it deviated significantly in Madras due to excess of heterozygotes (TBL.3).

5.5 GLUTAMATE DEHYDROGENASE (GDH)

Glutamate dehydrogenase was found highly polymorphic at its first locus in all the populations of Sardinella longiceps. Here allele frequencies were sufficiently different to consider each population as different. The significant allele frequency differences between populations of east and west coast and within east and west coast suggest genetic heterogeneity of all the five populations. The frequency of **F** allele of first locus ranged from 0.24 in Calicut to 0.90 in Madras. Similarly, the frequency of **F** allele of second locus ranged from 0 in Calicut and 1 in Cochin, Mangalore and Madras. It means that Calicut population had altogether different allele, namely **S**, in the place of **F** allele in other populations (Cochin, Mangalore and Madras). However, Mandapam population, though very far off from Cochin, showed same **F** allele as predominant (TBL.2).

Thus the second locus of GDH showed polymorphism in Mandapam population alone, whereas monomorphim in all other populations indicating

strong genetic differentiation of Mandapam population. Though Cochin, Calicut, Mangalore and Madras populations showed monomorphic alleles, strong genetic heterogeneity of Calicut population is indicated by its own cent percent **S** allele. Calicut population is uniquely different in having slow allele of second locus as fixed. Thus Calicut population is highly differentiated from that of nearby Cochin and Mangalore populations. Though the frequency of **F** allele was identical in Cochin, Mangalore and Madras, their genetic differences are also evident by the presence of a genetically different population between any two populations. For example, genetically different Calicut population becomes a genetic barrier between Cochin and Mangalore. The same is true between Cochin and Madras populations because of the polymorphic Mandapam population in between. The genetic differences between Mandapam and Madras were also indicated by a polymorphic second locus in Mandapam and a monomorphic second locus in Madras.

GDH has been reported as monomorphic in most of the fish species such as New Zealand snapper (Smith et al., 1978), Atlantic mackerel (Smith and Jamieson, 1980), walleye pollock (Grant and Utter, 1980), milk fish (Winans, 1980), black seabrem (Sumantadinata and Taniguchi, 1982), orange roughy (Smith, 1986), California bass (Graves et al., 1990), grey mullet (Vijayakumar, 1992). However, polymorphic GDH loci have been reported in blennius, Hypoblennius jenkinsi (Present, 1987). In his study, out of the three populations, only one showed polymorphism (frequency of a and b allele being 0.986 and 0.014 respectively), whereas other two populations showed cent percent a allele, a comparable phenomenon noticed in the present

investigation at GDH second locus. A comparable polymorphic and monomorphic nature of GDH enzyme locus was also reported in oyster drill (Liu et al., 1991), which was used as one of the genetic parameters for stock differentiation. It appears that this may be the first report of highly polymorphic GDH loci in any fish species.

Hardy-Weinberg equilibrium tests conducted, showed significant deviation in three out of the ten cases. Cochin and Mangalore populations showed excess of heterozygotes at GDH-I locus, whereas Mandapam population showed excess of homozygotes at GDH-II locus. Homozygote excess was reported at GDH enzyme locus in oyster drill (Liu et al., 1991).

5.6 ISOCITRATE DEHYDROGENASE (IDH)

In all the populations of S. longiceps tested for IDH, first locus in liver tissue showed monomorphism with a single invariant band, whereas second locus showed polymorphism. Brown (1991) observed such kind of monomorphism and polymorphism occurring in a single enzyme system at different loci in his study on genetic variation and population structure of blacklip abalone, Haliotis rubra. But instances of monomorphic IDH also is not uncommon in fishes. For example, monomorphic IDH loci in Northern pike (Seeb et al., 1987), two monomorphic loci in Pacific cod (Grant et al., 1987). Other reports of IDH monomorphism are in walleye pollock (Grant and Utter, 1980), paddle fish (Carlson et al., 1982), Catostomus (Buth and Crabtree, 1982) and Arctic charr (Magnusson and Fergusson, 1987).

Significant allele frequency differences for the major allele **F** was observed at IDH-II locus between Cochin, Mangalore, Madras group. it being

monomorphic and Calicut, Mandapam group, it being polymorphic. The presence of polymorphism at IDH-II locus only in Calicut and monomorphism at Cochin and Mangalore suggests a unique genetic difference of Calicut population on the west coast. The middle regional position of Calicut polymorphic population between Cochin and Mangalore monomorphic populations also causes a genetic barrier between Cochin and Mangalore populations, keeping latter populations isolated as already discussed elsewhere (ADH, GDH). This assumption is further strengthened by similar, but significant IDH allelic frequency difference between 2 local populations on the east coast, where major allele at Mandapam was **S** allele, while it was cent percent **F** allele in Madras. In otherwords, Mandapam population showed high polymorphism whereas Madras population showed just a monomorphic **F** allele. These differences also indicate strong genetic differences between the two east coast populations. Smith et al. (1979) detected five alleles(New Zealand hake), where one allele, IDH-III was in high frequency in all samples. They observed lower allelic frequencies in more southernly samples than those samples from around the mainland. Similar significant allele frequency differences have been reported by Altukhov and Salmenkova (1981) in fish populations of USSR. They have reported allele frequency differences for IDH in chumsalmon and Atlantic salmon. Winans (1980) also observed variation at IDH loci between southern and central Phillippine populations of milk fish, Chanos chanos.

Hardy-Weinberg equilibrium tests indicated excess of homozygotes at IDH-II locus in Mandapam population alone whereas balanced polymorphism

was observed in Calicut population. Similar unbalanced IDH polymorphic locus was also reported by Richardson (1982a) who observed excess of homozygotes in IDH enzyme in his study in jack mackerel. Brown (1991) also observed consistent heterozygote deficiency within the subpopulation of black lip abalone, Haliotis rubra. Therefore, the phenomenon of excess of homozygote at IDH locus appears to be a common phenomenon in marine fishes.

5.7 LACTATE DEHYDROGENASE (LDH)

Zymogram patterns of eyelens lactate dehydrogenase (LDH) in Sardinella longiceps collected from Cochin, Calicut, Mangalore, Mandapam and Madras (FIG.16,17, PLT.16,17) showed intraspecies polymorphisms. Though three loci were detected, only two were polymorphic. Such intraspecies LDH polymorphisms have been reported by many other authors (Utter and Hodgins, 1972; Altukhov, 1981; Andersson et al., 1983; Mork et al., 1985; Smith, 1986; Smith et al., 1990). The present observation of monomorphic and polymorphic LDH loci in the same tissue is also not uncommon in fishes (Ryman and Stahl, 1981; Campton and Utter, 1987). LDH-I locus was not polymorphic in any of the five populations sampled, whereas LDH-II locus showed significant allele frequency differences between Cochin (1.00)/Calicut (1.00)/Madras (1.00) and Mangalore (0.88)/Mandapam (0.66). Thus non-polymorphic Cochin, Calicut, Madras populations are genetically very different from polymorphic Mangalore and Mandapam populations. For similar reasons, monomorphic Calicut population is genetically different from polymorphic Mangalore population. For the same reason, Mandapam and Madras populations are genetically different, the former being polymorphic and latter monomorphic.

LDH-III locus also showed polymorphism in Cochin, Calicut, Madras and Mangalore, Mandapam populations. However, apparent allelic frequency differences were shown only by Mangalore/Mandapam populations. Such differences in allele frequencies have been reported by various investigators for LDH enzyme. For example, Utter and Hodgins (1972) distinguished a trout population on the basis of LDH heterogeneity. Mork et al. (1985) found out large statistically significant heterogeneity of allele frequencies between Atlantic cod populations. Significant allele frequency differences at an LDH-2 locus has been reported by Jorstad and Pederson (1986) in oceanic and fjord herring populations as reported by Smith et al. (1990).

Though LDH patterns from eyelens showed polymorphism and stock difference in S. longiceps tested in the present investigation, a comparison of various aspects of its nature in different populations is warranted. Comparatively high Chi-square value was observed in Mangalore population at LDH-II locus, due to significant deviation from Hardy-Weinberg equilibrium. Here, the excess of homozygotes was observed. Interestingly, excess of heterozygotes was observed in Mandapam population. Similarly, LDH-III locus also showed significant deviations from the expected values. Mangalore population showed excess of homozygotes whereas Mandapam population showed excess of heterozygotes. Both Cochin and Madras showed only heterozygous patterns and hence they showed equal frequencies. The only population which showed balanced polymorphism was Calicut population for both alleles and therefore the Chi-square value is 0.0015.

Excess of homozygotes or excess of heterozygotes were also reported at LDH locus in other fishes. Heterozygote excess has been reported by

Wright and Atherton (1970) in offspring generation of brook trout indicating heterozygote superiority in offspring generations. They found only 2 homozygotes and that too with almost equal frequencies. Daly and Richardson (1980) also observed lack of LDH heterozygotes in their study in anchovies. Ryman and Stahl (1981) could find out an apparent absence of heterozygotes at an LDH-locus in their study in salmonids.

The possible reason for the observation of excess of heterozygotes in Sardinella longiceps tested in the present case may be taken as due to heterozygote superiority at adult stage of oil sardine. However the observation of balanced polymorphism in Calicut population, inspite of the sample composed of 15-20 cm size range does not support the above assumption.

Though the LDH allele frequency values between Sardinella longiceps populations compared in the present investigation indicated genetic stock differences, further detailed comparison of these populations is desirable to explain the cent percent heterozygote in Cochin and Madras populations.

5.8 MALATE DEHYDROGENASE (MDH)

MDH zymogram patterns showed polymorphism at its first locus and monomorphism at its second locus. However, first locus showed monomorphism at Cochin, Calicut and Mandapam populations, whereas polymorphism at Mangalore and Madras. The predominant **F** allele frequency was 0.57 at Mangalore and 0.70 at Madras. The monomorphic populations showed cent percent **F** allele. Therefore, the polymorphism at Mangalore, Madras and monomorphism at Cochin, Calicut and Mandapam indicated that Mangalore and Madras

populations are genetically very different from that of Cochin, Calicut and Mandapam. For the same reason, polymorphic Mangalore population is genetically different from monomorphic Cochin and Calicut populations in the west coast. Similarly, monomorphic Mandapam population is genetically different from polymorphic Madras population in the east coast. In short, MDH I locus is a strong genetic marker to reveal the genetic stock differences among Calicut, Mangalore, Mandapam and Madras populations. The second locus was monomorphic in all the populations tested with cent percent **S** allele (TBL.2).

MDH has been reported as polymorphic in most of the fishes such as sockeye salmon (Grant et al., 1980), trout (Thompson, 1985), masu salmon (Okazaki, 1986), Atlantic herring (Grant, 1986) and barramundi (Salini and Shaklee, 1988). However, largest frequency differences among the stocks of white fish at MDH locus have been reported by Casselman et al. (1981). Similarly, the highly significant allele frequency differences at MDH locus were found among five stocks of Coregonus clupeaformis by Ihssen et al. (1981). The significance was shown by the MDH-3, 4 locus with allelic frequencies ranging from 0.175 to 0.660. Busak et al. (1980) found allele frequency differences at MDH-2 locus among six trout populations. Out of the six populations, three showed cent percent 100 allele and one population showed 0.986 for the most common allele. However, two populations showed only 0.086 and 0.033, whereas another allele 130 was in higher frequency (0.914 and 0.967 respectively). Campton and Utter (1987) could find out significant allele frequency difference at MDH-3, 4 loci among the populations

of cutthroat trout. The range of allele frequency was from 0.58 to 0.94. Thus the present findings of the polymorphic nature of MDH are comparable in many respects with that of other fish species reported by the above authors. The monomorphic and polymorphic nature of MDH is a common phenomenon in many of the fish species thus investigated. Besides, the degree of polymorphism ranging from cent percent allele at a particular locus to highly polymorphic range at another allele was also similar to the type reported in the present investigation. The range of allele frequencies between populations were considerably different to indicate strong genetic stock differences in many of these fish species investigated, indicating that MDH enzyme system is an excellent genetic marker for population genetic studies.

Hardy-Weinberg equilibrium tests conducted showed good agreement between observed and expected values indicating a balanced polymorphism in both polymorphic stations (TBL.3).

5.9 XANTHINE DEHYDROGENASE (XDH)

Zymogram patterns of XDH in heart tissue in Sardinella longiceps collected from Cochin, Calicut, Mangalore, Mandapam and Madras showed polymorphism at XDH-I locus and monomorphism at XDH-II locus (FIG.19 PLT.19). In the polymorphic locus **F** was the predominant allele, the frequency of which ranged from 0.54 to 0.92 indicating significant differences between the populations tested (TBL.2). The highest range occurred between Calicut and Cochin, followed by between Cochin/Madras and Mandapam/Mangalore. Besides, the allele frequencies of Madras, Mandapam and Mangalore were closely comparable. The second locus was totally monomorphic in all the

populations tested with cent percent **S** allele. Thus XDH first locus is a good genetic marker to differentiate genetic stock differences existing among all the three west coast populations, but not that of the east coast.

XDH has been reported as monomorphic in most of the fishes namely trout (Thompson, 1985), orange roughy (Smith, 1986), Atlantic halibut (Fevolden and Haug, 1988), sharks (Lavery and Shaklee, 1989), California bass (Graves et al., 1990) and oyster drill (Liu et al., 1991). However, a single polymorphic XDH locus was reported in Atlantic mackerel (Smith and Jamieson, 1978). They reported similar allele frequencies throughout their sampling ranges. Fujio et al. (1983) reported polymorphism in five species of marine molluscs out of the twenty tested. Similarly, Berglund and Lagercrantz (1983) observed different allele fixation in two species of Palaemon prawn species. Thus the present report of two loci with one polymorphic and another monomorphic, the former having significant allele frequency differences between populations appears to be first of its kind. In the present study, Mangalore and Mandapam, two distant populations showed same allele frequencies suggesting its genetic similarity. Similar cases have been reported by many investigators (Grant, 1984; Grant and Utter, 1984; Ryman et al., 1984). Similarly, Milton and Shaklee (1987) reported genetic similarity over long distances as a characteristic of many marine teleost species.

However, the presence of genetically different other stocks such as Cochin and Calicut between Mangalore and Mandapam creats a genetic barrier to their mixing during breeding season.

Deviation of genotype distribution from that of expected was not significant at Cochin, but nearly significant at Mangalore, Mandapam and Madras. It was very significant at Calicut. Significance was mainly due to excess of heterozygotes as in other loci discussed in other sections.

All the results of the present investigation evaluated through the above discussions may be summarised as follows: Zymogram patterns of nine enzyme systems, namely, ADH, AO, EST, G6PD, GDH, IDH, LDH, MDH and XDH was found to be polymorphic in the species, Sardinella longiceps. However, certain locus (or loci) of specific enzyme was polymorphic in one or more populations while monomorphic in one or more populations as the case may be, suggesting population specific differences in the nature of polymorphism. For example, ADH-I and II loci were polymorphic in Calicut and Mandapam while these were monomorphic in Cochin, Mangalore and Madras. AO-III was polymorphic in Cochin, while it was monomorphic in Calicut, Mangalore, Mandapam and Madras. Esterase-I was polymorphic in Cochin. Calicut, Mangalore, while monomorphic in Mandapam and Madras. On the otherhand, G6PD-III was monomorphic in the west coast populations, while polymorphic in the east coast populations. Thus EST-I and G6PD-III clearly revealed the genetic differences of S. longiceps from west and east coast. Again two loci of G6PD (I and II) were monomorphic in all the five populations while third locus was polymorphic in Mandapam and Madras populations, thus a single enzyme could reval the genetic stock differences between east and west populations of S. longiceps. Another important aspect of genetic stock differences of five populations of S. longiceps was, significant differences

in the allelic frequencies of polymorphic loci themselves. For example, ADH-II frequency of **S** allele was 0.95 in Cochin, while it was 0.75 in Mandapam. Frequency of **S** allele of AO-II was 0.31 in Cochin, while 0.73 in Mandapam and 0.42 in Madras. Frequency of **S** allele of EST-II was 0.50 in Cochin, 0.17 in Calicut, 0.84 in Mangalore and 0.60 in Mandapam and that of EST-III, 0.38 in Cochin, 0.56 in Calicut, 0.21 in Mandapam and 0.60 in Madras. Thus allelic frequency difference at EST-III locus alone could show genetic stock differences of four out of five populations tested. Similar significant differences among all the five populations except one were also shown by a single locus of GDH-I, the frequencies of **S** being 0.36 (Cochin), 0.76 (Calicut), 0.46 (Mangalore) and 0.16 (Mandapam). Other enzyme loci that showed allelic frequency differences are EST-IV, IDH-II, LDH-II, III, MDH-I and XDH-I. The degree of deviations between observed and expected genotype frequencies at polymorphic loci was significant only at 24 out of 125 cases tested.

Table: 2

Allele frequencies at 25 loci in five populations of Sardinella longiceps

Sl. No.	Locus	Allele	Populations				
			CHN	CCT	MRE	MDM	MAS
1.	ADH I	S	1.00	0.75	1.00	0.73	1.00
		F	0.00	0.25	0.00	0.27	0.00
2.	ADH II	S	1.00	0.95	1.00	0.75	1.00
		F	0.00	0.05	0.00	0.25	0.00
3.	AO I	S	1.00	1.00	1.00	1.00	1.00
4.	AO II	S	0.31	0.50	0.50	0.73	0.42
		F	0.69	0.50	0.50	0.27	0.58
5.	AO III	S	0.19	0.00	0.00	1.00	1.00
		F	0.81	1.00	1.00	0.00	0.00
6.	EST I	S	0.76	0.73	0.93	1.00	1.00
		F	0.24	0.27	0.07	0.00	0.00
7.	EST II	S	0.50	0.17	0.84	0.60	0.75
		F	0.50	0.83	0.16	0.40	0.25
8.	EST III	S	0.38	0.56	0.58	0.21	0.60
		F	0.62	0.44	0.42	0.79	0.40
9.	EST IV	S	0.62	0.60	0.90	0.54	0.73
		F	0.38	0.40	0.10	0.46	0.27
10.	G6PD I	S	1.00	1.00	1.00	1.00	1.00
11.	G6PD II	S	1.00	1.00	1.00	1.00	1.00
12.	G6PD III	S	1.00	1.00	1.00	0.24	0.47
		F	0.00	0.00	0.00	0.76	0.53
13.	G6PD IV	S	1.00	1.00	1.00	1.00	1.00
14.	G6PD V	S	1.00	1.00	1.00	1.00	1.00
15.	GDH I	S	0.36	0.76	0.46	0.16	0.10
		F	0.64	0.24	0.54	0.84	0.90

Table: 2 contd...

Sl. No.	Locus	Allele	Populations				
			CHN	CCT	MRE	MDM	MAS
16.	GDH II	S	0.00	1.00	0.00	0.68	0.00
		F	1.00	0.00	1.00	0.32	1.00
17.	IDH I	S	1.00	1.00	1.00	1.00	1.00
18.	IDH II	S	0.00	0.09	0.00	0.63	0.00
		F	1.00	0.91	1.00	0.37	1.00
19.	LDH I	S	1.00	1.00	1.00	1.00	1.00
20.	LDH II	S	0.00	0.00	0.16	0.34	0.00
		F	1.00	1.00	0.84	0.66	1.00
21.	LDH III	S	0.50	0.54	0.36	0.32	0.50
		F	0.50	0.46	0.64	0.68	0.50
22.	MDH I	S	0.00	0.00	0.43	0.00	0.30
		F	1.00	1.00	0.57	1.00	0.70
23.	MDH II	S	1.00	1.00	1.00	1.00	1.00
24.	XDH I	S	0.08	0.46	0.29	0.29	0.32
		F	0.92	0.54	0.71	0.71	0.68
25.	XDH II	S	1.00	1.00	1.00	1.00	1.00

6. GENERAL DISCUSSION

6.1 HARDY-WEINBERG EQUILIBRIUM

Hardy-Weinberg law is the corner stone of the modern population genetic studies. It describes the constant equilibrium condition of gene frequencies in a sexually reproducing population, enabling one, prediction of distribution of genotype proportions (Dobzhansky, 1967). Thus the expected genotypic frequencies in the test samples can be estimated on the basis of observed genotype frequencies under the assumption of random mating of the population from which the sample was drawn. In other words, the test helps in assessing the genetic nature of the observed phenotypes and the degree of deviations from that of expected proportions (Ayala and Kiger, 1980; Utter et al., 1987). The Hardy-Weinberg law implies equilibrium condition between observed and expected gene frequencies in an ideal population which is free from factors like mutation, selection, migration, genetic drift etc. However, in practice, significant deviations from equilibrium condition due to excess of homozygotes or/and heterozygotes are not rare in genetic studies of fishes and shell fishes (Peterson and Shehadeh, 1971; Utter and Hodgins, 1972; Daly and Richardson, 1980; Imhof et al., 1980; Dehring et al., 1981; Buth and Crabtree, 1982; Richardson, 1982a; Stahl, 1987; Verspoor, 1988; Lavery and Shaklee, 1989; Hernandez-Martich and Smith 1990; Vijayakumar, 1992).

In the present investigation, a comparison of the observed and expected genotype distributions in S. longiceps showed significant deviations in only twenty four cases out of the one hundred and twenty five tested (TBL.3).

Table : 3

Comparison of observed and expected (Hardy-Weinberg Law) genotype frequencies in *Sardinella longiceps* populations

Sl. No.	Locus	Geno Type	COCHIN			CALICUT			MANGALORE			MANDAPAM			MADRAS		
			OBS	EXP	CHI	OBS	EXP	CHI	OBS	EXP	CHI	OBS	EXP	CHI	OBS	EXP	CHI
1.	ADH-I	SS	34	34	0	10	11.25	0.14	22	22	0	10	11.60	0.22	25	25	0
		SF	0	-	-	10	7.50	0.83	0	-	-	12	8.73	1.22	0	-	-
		FF	0	-	-	0	1.25	1.25	0	-	-	0	1.64	1.64	0	-	-
2.	ADH-II	SS	34	34	0	18	18.05	0.0001	22	22	0	10	11.25	0.14	20	20	0
		SF	0	-	-	2	1.90	0.005	0	-	-	10	7.50	0.83	0	-	-
		FF	0	-	-	0	0.05	0.05	0	-	-	0	1.25	1.25	0	-	-
3.	AO-I	SS	48	48	0	24	24	0	24	24	0	20	20	0	19	19	0
		SF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
		FF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
4.	AO-II	SS	0	4.69	4.69	0	6	6	0	6	6	9	10.50	0.21	6	3.37	2.05
		SF	30	20.63	4.26	24	12	12	24	12	12	11	7.80	1.31	4	9.26	2.99
		FF	18	22.69	0.97	0	6	6	0	6	6	0	1.51	1.51	9	6.37	1.09
5.	AO-III	SS	0	1.69	1.69	0	-	-	0	-	-	20	20	0	19	19	0
		SF	18	14.60	0.79	0	-	-	0	-	-	0	-	-	0	-	-
		FF	30	31.7	0.09	24	24	0	24	24	0	0	-	-	0	-	-
6.	EST-I	SS	19	21.19	0.23	10	11.64	0.23	19	19.10	0.0005	24	24	0	24	24	0
		SF	18	13.62	1.41	12	8.73	1.22	3	2.80	0.014	0	-	-	0	-	-
		FF	0	2.19	2.19	0	1.64	1.64	0	0.10	0.10	0	-	-	0	-	-

Table : 3 Contd...

Sl. No.	Locus	Geno Type	COCHIN			CALICUT			MANGALORE			MANDAPAM			MADRAS		
			OBS	EXP	CHI	OBS	EXP	CHI	OBS	EXP	CHI	OBS	EXP	CHI	OBS	EXP	CHI
7.	EST-II	SS	3	9	4	0	0.67	0.67	16	15.60	0.01	7	7.20	0.006	14	11.25	0.67
		SF	30	18	8	8	6.67	0.27	5	5.89	0.13	10	9.60	0.02	2	7.50	4.03
		FF	3	9	4	16	16.7	0.03	1	0.56	0.04	3	3.20	0.01	4	1.25	6.05
8.	EST-III	SS	0	3.37	3.37	8	7.59	0.02	11	8.16	0.99	2	1.04	0.89	9	8.76	0.01
		SF	18	11.25	4.75	11	11.81	0.06	6	11.70	2.78	6	7.92	0.47	11	11.48	0.02
		FF	6	9.37	1.21	5	4.50	0.04	7	4.14	1.92	16	15.04	0.06	4	3.76	0.02
9.	EST-IV	SS	12	9.85	0.47	9	8.76	0.01	19	19.26	0.004	4	6.79	1.15	12	12.80	0.05
		SF	8	12.30	1.50	11	11.48	0.02	5	4.50	0.05	17	11.41	2.74	11	9.48	0.24
		FF	6	3.84	1.22	4	3.76	0.02	0	0.26	0.26	2	4.76	1.63	1	1.76	0.33
10.	G6PD-I	SS	48	48	0	24	24	0	21	21	0	17	17	0	24	24	0
		SF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
		FF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
11.	G6PD-II	SS	48	48	0	24	24	0	21	21	0	17	17	0	24	24	0
		SF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
		FF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
12.	G6PD-III	SS	48	48	0	24	24	0	21	21	0	0	0.94	0.94	0	5.30	5.30
		SF	0	-	-	0	-	-	0	-	-	8	6.11	0.59	23	11.95	10.22
		FF	0	-	-	0	-	-	0	-	-	9	9.93	0.09	1	6.74	4.89

Table : 3 Contd...

Sl. No.	Locus	Geno Type	COCHIN			CALICUT			MANGALORE			MANDAPAM			MADRAS		
			OBS	EXP	CHI	OBS	EXP	CHI	OBS	EXP	CHI	OBS	EXP	CHI	OBS	EXP	CHI
13.	G6PD-IV	SS	48	48	0	24	24	0	21	21	0	17	17	0	24	24	0
		SF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
		FF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
14.	G6PD-V	SS	48	48	0	24	24	0	21	21	0	17	17	0	24	24	0
		SF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
		FF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
15.	GDH-I	SS	1	5.03	3.23	12	13.3	0.13	0	5.04	5.04	0	0.47	0.47	0	0.20	0.20
		SF	26	17.90	3.67	11	8.36	0.83	22	11.92	8.53	6	5.05	0.18	4	3.6	0.04
		FF	12	16.03	1.01	0	1.32	1.32	2	7.04	3.61	13	13.47	0.02	16	16.2	0.003
16.	GDH-II	SS	0	-	-	23	23	0	0	-	-	13	8.89	1.90	0	-	-
		SF	0	-	-	0	-	-	0	-		0	8.21	8.21	0	-	-
		FF	39	39	0	0	-	-	24	24	0	6	1.89	8.94	20	20	0
17.	IDH-I	SS	48	48	0	72	72	0	24	24	0	30	30	0	24	24	0
		SF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
		FF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
18.	IDH-II	SS	0	-	-	1	0.49	0.53	0	-	-	16	12	1.33	0	-	-
		SF	0	-	-	9	10.02	0.10	0	-	-	6	13.9	4.49	0	-	-
		FF	48	48	0	52	51.5	0.01	24	24	0	8	4.04	3.89	24	24	0
19.	LDH-I	SS	48	48	0	24	24	0	24	24	0	24	24	0	24	24	0
		SF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
		FF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-

Table : 3 Contd...

Sl. No.	Locus	Geno type	COCHIN			CALICUT			MANGALORE			MANDAPAM			MADRAS		
			OBS	EXP	CHI	OBS	EXP	CHI	OBS	EXP	CHI	OBS	EXP	CHI	OBS	EXP	CHI
20.	LDH-II	SS	0	-	-	0	-	-	3	0.56	10.70	0	2.22	2.22	0	-	-
		SF	0	-	-	0	-	-	1	5.89	4.06	13	8.55	2.32	0	-	-
		FF	48	48	0	24	24	0	18	15.50	0.40	6	8.22	0.60	24	24	0
21.	LDH-III	SS	0	10.5	10.5	7	7.04	0.0002	8	2.91	8.90	0	1.89	1.89	0	6.0	6.0
		SF	42	21.0	21.0	12	11.90	0.001	0	10.18	10.18	12	8.21	1.75	24	12.0	12.0
		FF	0	10.5	10.5	5	5.04	0.003	14	8.91	2.91	7	8.89	0.40	0	6.0	6.0
22.	MDH-I	SS	0	-	-	0	-	-	6	4.35	0.63	0	-	-	1	1.8	0.36
		SF	0	-	-	0	-	-	8	11.30	0.96	0	-	-	10	8.4	0.31
		FF	48	48	0	48	48	0	9	7.35	0.37	48	48	0	9	9.8	0.07
23.	MDH-II	SS	48	48	0	48	48	0	48	48	0	48	48	0	48	48	0
		SF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
		FF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
24.	XDH-I	SS	0	0.36	0.36	0	4.8	4.8	0	2.04	2.04	0	2.04	2.04	0	1.89	1.89
		SF	9	8.28	0.06	21	11.40	8.08	14	9.92	1.68	14	9.92	1.68	12	8.21	1.75
		FF	47	47.36	0.0003	2	6.80	3.39	10	12.04	0.35	10	12.04	0.35	7	8.89	0.40
25.	XDH-II	SS	56	56	0	23	23	0	24	24	0	24	24	0	19	19	0
		SF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
		FF	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-

The significant deviations were mainly due to excess of heterozygotes in seventeen cases while seven cases due to excess of homozygotes (TBL.6). Reports on the phenomenon of excess of heterozygotes in fishes are available. For example, Peterson and Shehadeh (1971) reported excess of heterozygotes in Mugil cephalus populations. Daly and Richardson (1980) also observed excess of heterozygotes in Anchovies. Excess of heterozygotes was also reported in lake white fish, Coregonus clupeaformis (Imhof et al., 1980). Similarly, Lavery and Shaklee (1989) observed four cases of excess of heterozygotes in shark, where the P values ranged from $P < 0.05$ to $P > 0.01$. A recent report on excess of heterozygotes is that of Vijayakumar (1992) in Mugil cephalus populations.

The examples of homozygote excess have also been reported by many investigators. Excess of homozygotes were observed in milk fish (Winans, 1980), mackerel (Smith et al., 1981a; Richardson, 1982a, Menezes et al., 1990), Catostomus (Buth and Crabtree, 1982), orange roughy (Smith, 1986), Hypoblennius (Present, 1987), cod (Grant et al., 1987), cat fishes (Suzuki and Phan, 1990), Mugil cephalus (Vijayakumar, 1992), marine molluscs (Tracey et al., 1975; Singh and Green, 1984; Zouros and Foltz, 1984) and marine prawns (Richardson, 1982c, Philip Samuel, 1987).

Interestingly, in the present study, only seven out of twenty four cases showed excess of homozygotes. Besides, homozygote excess was not observed in Cochin and Calicut populations whereas Mandapam and Madras populations showed two cases each and three at Mangalore (TBL.6). The P values were between $P < 0.01$ to $P > 0.001$ with degree of freedom one

Table: 6

Excess of Homozygotes/Heterozygotes at significant level in Sardinella longiceps populations

Sl. No.	Locus	Cochin		Calicut		Mangalore		Mandapam		Madras	
		HOM	HET	HOM	HET	HOM	HET	HOM	HET	HOM	HET
1.	ADH I	-	-	-	-	-	-	-	-	-	-
2.	ADH II	-	-	-	-	-	-	-	-	-	-
3.	AO I	-	-	-	-	-	-	-	-	-	-
4.	AO II	-	+	-	+	-	+	-	-	-	-
5.	AO III	-	-	-	-	-	-	-	-	-	-
6	EST I	-	-	-	-	-	-	-	-	-	-
7.	EST II	-	+	-	-	-	-	-	-	-	-
8.	EST III	-	+	-	-	+	-	-	-	-	-
9.	EST IV	-	-	-	-	-	-	-	+	-	-
10.	G6PD I	-	-	-	-	-	-	-	-	-	-
11.	G6PD II	-	-	-	-	-	-	-	-	-	-
12.	G6PD III	-	-	-	-	-	-	-	-	-	-
13.	G6PD IV	-	-	-	-	-	-	-	-	-	-
14.	G6PD V	-	-	-	-	-	-	-	-	-	-
15	GDH I	-	+	-	-	-	+	-	-	-	-
16.	GDH II	-	-	-	-	-	-	+	-	-	-
17.	IDH I	-	-	-	-	-	-	-	-	-	-
18.	IDH II	-	-	-	-	-	-	+	-	-	-
19.	LDH I	-	-	-	-	-	-	-	-	-	-
20.	LDH II	-	-	-	-	+	-	-	+	-	-
21.	LDH III	-	+	-	-	+	-	-	+	-	-
22.	MDH I	-	-	-	-	-	-	-	-	-	-
23.	MDH II	-	-	-	-	-	-	-	-	-	-
24.	XDH I	-	-	-	+	-	+	-	+	-	+
25.	XDH II	-	-	-	-	-	-	-	-	-	-
	Total	0	5	0	2	3	3	2	4	2	3

\+ = Excess of Homozygotes/Heterozygotes

at about seven loci distributed among five enzyme systems namely aldehyde oxidase, esterase, glutamate dehydrogenase, isocitrate dehydrogenase and lactate dehydrogenase.

Though the possible reasons given for the occurrence of excess of heterozygotes in the above examples are, as usual, presumptive rather than experimental proofs, some of them are discussed below. Stoneking et al. (1981) reported that excess of heterozygotes can occur due to duplication of locus as explained by Dehring et al. (1981), whereas Stahl (1987) stated that it may be due to small number of parents during breeding process or due to possible statistical type I errors. Meanwhile, excess of heterozygotes may occur due to the effect of larger year class spawned when there is more interbreeding between populations (Imhof et al., 1980). Similarly effect of hatchery procedures can also cause excess of heterozygotes (Stahl, 1987; Verspoor, 1988).

Ponniah (1989) reviewed reports on excess of heterozygotes in fishes and their apparent correlations with different environmental parameters. The review reveals the complexity of probable mechanisms causing excess of heterozygotes at different loci such as esterase, lactate dehydrogenase, transferin, phosphoglucomutase and glycerophosphate dehydrogenase in different fish species. The phenomenon of excess of heterozygosity in the abyssal population of fish, Sebastodes alutus at phosphoglucomutase and glyceraldehyde-phosphate dehydrogenase loci and lack of such a phenomenon in shallow water populations have been observed by Johnson et al. (1970) as explained in the

review (Ponniah, 1989). Similarly, Gooch and Schopf (1971) found relation between heterozygote excess and depth and pollution. In their study in ectoproct, they stated that excess of heterozygotes in shallowest water may indicate that heterozygotes have superior tolerance to the relatively more variable zone just below the surface. Vijayakumar (1992) observed excess of heterozygotes in nineteen out of the twenty four cases of deviations and suggested that some form of heterozygous advantage exists in Mugil cephalus populations causing the unequilibrium condition in genotype distribution.

The present observation of significant cases of deviation from Hardy-Weinberg equilibrium condition in the genotype distribution in populations of Sardinella longiceps cannot be due to any of the above discussed non-genetic conditions such as differences of depth, pollution, sex or size of specimens. Because the population samples tested in the present study have all come from a particular depth range and uniform environmental conditions. This argument is further strengthened by the fact that genetic stock differences were detected even between nearby regions, similar in environmental conditions and depth etc. That is, populations of Cochin, Calicut and Mangalore were found to be genetically different, though all these three populations belong to west coast of India. Similarly, the two populations drawn from Mandapam and Madras were also found to be genetically heterogeneous, inspite of comparable ecological conditions of the east coast. Again, the observation of excess or deficiency of different genotypes cannot be due to differences of sex or size of the sample specimens used for the present study, because the range of size was minimal, it being 15-20 cms containing both sexes. Above all, a specific observed condition in a particular species like S. longiceps

cannot be proved or disproved, justified or unjustified on the basis of findings observed in other species and merely explained on presumptive basis, as done in most of the explanations given in the above discussed reports.

The possible reasons for the observation of excess of heterozygotes in the present study may be that a hybrid vigour is produced even in natural conditions of heterozygote formation, as also observed in M. cephalus (Vijayakumar, 1992). Moreover, for a highly mobile pelagic fish like S. longiceps, the excess of heterozygotes may be a natural phenomenon.

A number of reasons have been attributed to the phenomenon of excess of homozygotes by various investigators (Hedgecock, 1977; Smith and Jamieson, 1978, 1980 and Grant et al., 1987). Smith et al. (1981a) reported that excess of homozygotes or heterozygote deficiency may be due to population mixing, known as Wahlund effect or it can be a reflection of narrow sampling range between two major populations. In their study in mackerel, breakdown of data by sex, year class, size, hauls etc. does not significantly reduce the excess of homozygotes. They have also reported that inbreeding could produce an excess of homozygote but is unlikely in a highly mobile and abundant species with external fertilization and a pelagic larval stage. Milkman and Beaty (1970) reported a substantial deficiency of heterozygotes in Mytilus edulis and they hypothesized that the deficiency might be due to the presence of a null allele or silent allele. Alleles of this type exhibit no activity or bands during staining procedures and therefore heterozygotes with non active alleles may be misscored as homozygotes. Koehn and Mitton (1972) also reported heterozygote deficiency and attributed the deficiency to selection.

Similarly, Koehn et al. (1973) revealed homozygote excess which was greater among small individuals and attributed these differences to microhabitat adaptation. Statistically significant deficiency of heterozygotes is usually interpretted as a mixture of two or more genetically differentiated populations (Skaala and Jorstad, 1987). However, genetic imbalances may be due to various aspects such as stock mixing, natural selection or drift in natural populations and therefore it needs a further study (Smith, 1990).

Though different possible reasons for excess of homozygotes in different reports are available and have been discussed above; they cannot be taken for granted as reasons for excess of homozygotes in S. longiceps in the present study. It is clear that out of one hundred and twenty five tests conducted for Hardy-Weinberg equilibrium only seven tests were found to be out of equilibrium due to excess of homozygotes. Interestingly, the rest seventeen cases show excess of heterozygotes. As cases of heterozygote excess are overwhelming in S. longiceps, the presence of few cases of excess homozygotes in the same species could be due to some other unknown factors controlled by ecological or other such factors as also reported in the case of M. cephalus (Vijayakumar, 1992).

The present observation of deviations from Hardy-Weinberg equilibrium condition cannot be due to any artifacts produced at any stages of electrophoretic analysis. Because, standardised procedures were followed throughout the experiment. These deviations cannot be also due to non-genetic or ontogenetic expressions of the enzyme systems considered in the present investi-

gation. Because, genetic basis of these enzymes has been well known. Besides, the expected genotype distribution patterns of the observed variant phenotypes were clearly shown in majority of the cases examined. Hence, these deviations must be part of natural phenomenon existing in the species like S. longiceps. Thus, these deviations certainly cannot affect the interpretations made and conclusions drawn from the electrophoretic data of the present investigation.

6.2 HETEROZYGOSITY

The degree of genetic variability can be better expressed by the rate of heterozygosity (Wishard et al., 1980; Ayala and Kiger, 1980). The average heterozygosity value of 0.21 (TBL.5) in the present study on oil sardine, Sardinella longiceps is comparable to the heterozygosity values quoted for several other marine species. However, the average heterozygosity value in the present study is comparatively higher than reported in many species of fishes. From different reports on average heterozygosity, it is evident that the values vary much from 0.001 (Seeb et al., 1987) to 0.36 (Vijayakumar, 1992). The low values of heterozygosities reported in different fish species are 0.075 in milk fish (Winans, 1980), 0.088-0.104 in sprat (Smith and Robertson, 1981), 0.08-0.73 in cutthroat trout (Campton and Utter, 1987), 0.02-0.04 in cod (Grant et al., 1987), 0.092 in rainbow trout (Berg and Gall, 1988) and 0.029 in barramundi (Salini and Shaklee, 1988).

There are also reports of higher heterozygosity values like 0.180 in Killifish (Mitton and Koehn, 1975), 0.17 in flatfish (Ward and Galleguillose,

Table: 5

Average Heterozygosity for the nine different enzymes of the five populations of Sardinella longiceps

Sl. No.	Enzyme	Average Heterozygosity per population					Average Heterozygosity per enzyme
		CHN	CCT	MRE	MDM	MAS	
1.	ADH	0.00	0.30	0.00	0.52	0.00	0.17
2.	AO	0.33	0.33	0.33	0.18	0.07	0.25
3.	EST	0.59	0.45	0.21	0.37	0.25	0.37
4.	G6PD	0.00	0.00	0.00	0.09	0.19	0.06
5.	GDH	0.33	0.24	0.46	0.16	0.10	0.26
6.	IDH	0.00	0.07	0.00	0.10	0.50	0.14
7.	LDH	0.33	0.17	0.02	0.44	0.33	0.26
8.	MDH	0.00	0.00	0.17	0.00	0.25	0.09
9.	XDH	0.08	0.46	0.29	0.29	0.32	0.29
	Total	1.66	2.02	1.48	2.15	2.01	
Average Heterozygosity in the five populations		0.18	0.22	0.16	0.24	0.22	
	Heterozygosity in Sardinella longiceps					0.21	

1977), 0.118 in plaice (Ward and Beardmore, 1977), 0.181 in eel (Rodino and Comparini, 1978), 0.129 in marine molluscs (Fujio et al., 1983), 0.121 in Penaeus japonicus (De Matthaeis et al., 1983) and 0.102 and 0.107 in salmon (Utter et al., 1989). A heterozygosity value of 0.33 was reported in the European hake (Mangaly and Jamieson, 1978). Recently a still higher heterozygosity value of 0.36 was reported in Indian striped mullet, Mugil cephalus (Vijayakumar, 1992). However, Powell (1975) reported Teleost heterozygosity range as 0.005 to 0.180 for over thirty one species from diverse environments. Moreover, Nevo (1978) reported mean heterozygosity as 0.0494 and also indicated that higher heterozygosity values may be expected in tropical vertebrates. Therefore, the present value of 0.21 may appear as slightly higher when compared to that of Powell (1975) whereas its comparison with that of Nevo (1978) may appear as much higher. Besides, when a comparison is made with that of M. cephalus (Vijayakumar, 1992) or European hake (Mangaly and Jamieson, 1978) it seems that the value of the present investigation is within the limits and not unusual. The range of heterozygosity values reported in the above discussion being 0.005 (Powell, 1975) to 0.36 (Vijayakumar, 1992), it is illogical and unscientific to attempt to judge the correctness of heterozygosity value of 0.21 reported in the present investigation. The value may be just specific and natural to the species S. longiceps. Neverthless, study of more loci in future investigation may bring down the present 0.21 heterozygosity value to a lower level.

It is seen that in biochemical population genetics, differences in heterozygosity values reported by different investigators often reveal the

sensitivity of this measure to the number and type of loci examined (Gorman and Renzi, 1979) as reported by Crozier (1987). A typical example of such variation in heterozygosity estimate according to the loci analysed is seen in deep water Macrurid, Corphaeinoides acrolepis (Somero and Soule, 1974). They examined six loci in this species and a comparatively high heterozygosity of 0.11 was reported. However, another study of twenty five loci in the same species showed a low value of 0.03 (Siebenaller, 1977). While discussing probable reasons for range in heterozygosity values, Smith and Jamieson (1980) indicated possible role of various parameters like, size and mobility of animal (Selander and Kaufman, 1973), size and age of the population (Soule, 1976), time of divergence (Somero and Soule, 1974), trophic stability (Valentine and Ayala, 1974) and environmental heterogeneity (Nevo, 1978). The probable reasons reported by Smith and Jamieson (1980) for varying heterozygosity values were again based on different nature or aspects of the enzyme concerned such as non-enzymatic and enzymatic proteins or glucose and non-glucose metabolising enzymes with different number of loci or even internal and external or single and multiple substrates etc. In addition to this, many investigators have found enzyme structure as more important determinant of heterozygosity levels, rather than their function. For example, monomeric enzymes are more variable than dimeric enzymes which in turn are more variable than tetrameric enzymes (Zouros, 1976; Ward, 1977; Ward and Gulle-guillose, 1977). Interestingly, Smith and Jamieson (1980) also have discussed about values on hypothetical conditions stated above for high and low genetic heterozygosity which were again found contradicting between species. Various

reasons have been attributed for observing high heterozygosity in P. japonicus such as large effective population size, genetic admixture, adaptive strategy at species or population levels (De Matthaeis et al., 1983).

The reason for observing the kind of average heterozygosity values in S. longiceps ranging from 0.16 (Mangalore) to 0.24 (Mandapam) cannot be due to the influence of size, sex, maturity stages, environmental parameters such as depth, geographic locations etc., because the samples collected contained specimens of both sexes and of uniform size range and of comparable environmental conditions. Therefore, the present observed values should be due to species specific and population specific conditions. According to Ayala and Kiger (1980) an electrophoretic survey of about 20 loci is usually sufficient to reveal the actual heterozygosity in the species. Therefore, the present observation of heterozygosity value of 0.21 in twenty five loci in S. longiceps is not unusual and may be natural for such a highly mobile, tropical pelagic clupeid fish. Besides, high heterozygosities can occur or can be a special feature of any pelagic clupeid or in general any small mobile fish (Selander and Kaufman, 1973; Valentine, 1976). Also, in general, varying heterozygosity values have been reported for species of different evolutionary origin and habitat specialism (Smith and Fujio, 1982) as reported by Crozier (1987). Besides, most of the enzymes and their loci are polymorphic in S. longiceps whereas polymorphism is of lesser order in other species reported by others and naturally heterozygosity is bound to be higher in this species. Above all, statistically similar values, particularly in terms of genetic variability cannot be expected in all fish species, because it may vary according

to the species concerned, loci tested, evolutionary pattern it followed and a number of still other unknown factors.

The above detailed general discussion proves one fact beyond doubt that a particular species can possess its own heterozygosity nature irrespective of geographic regions and number of protein loci examined. It is beyond the scope of the objectives of the present investigation to seek and explain the reasons for the type of heterozygosity observed in S. longiceps tested in the present investigation. The important question is, how the heterozygosity values can probably contribute in achieving the objectives of the present investigation. In this respect, it may be helpful to compare interpopulational heterozygosity in the species investigated as an additional indication of genetic composition of the species. The Table 5 shows average heterozygosity values for twenty five loci examined among five populations of S. longiceps. It ranged from 0.16-0.24. The difference between these two ranges is 0.08 a value much higher than 0.048 reported for eighty two fish species (Winans, 1980) and it further suggests the heterogeneity of the populations tested. The highest difference of average interpopulational heterogeneity indicated by heterozygosity occurred between Mangalore (0.16) and Mandapam (0.24). Comparable heterozygosity differences, but of lesser order, occurred between any other two populations such as Cochin, Calicut and Madras (TBL.4) corroborating basic differences in the genetic compositions of these populations as already indicated by differences of allelic frequencies and values of genetic identity and distance discussed elsewhere.

Table: 4

Heterozygosity at 25 loci in five populations of *Sardinella longiceps*

Sl. No.	Locus	Heterozygosity				
		CHN	CCT	MRE	MDM	MAS
1.	ADH I	0.00	0.50	0.00	0.55	0.00
2.	ADH II	0.00	0.10	0.00	0.50	0.00
3.	AO I	0.00	0.00	0.00	0.00	0.00
4.	AO II	0.63	1.00	1.00	0.55	0.21
5.	AO III	0.38	0.00	0.00	0.00	0.00
6.	EST I	0.49	0.55	0.14	0.00	0.00
7.	EST II	0.83	0.33	0.28	0.50	0.10
8.	EST III	0.75	0.49	0.25	0.25	0.49
9.	EST IV	0.31	0.49	0.21	0.74	0.49
10.	G6PD I	0.00	0.00	0.00	0.00	0.00
11.	G6PD II	0.00	0.00	0.00	0.00	0.00
12.	G6PD III	0.00	0.00	0.00	0.47	0.96
13.	G6PD IV	0.00	0.00	0.00	0.00	0.00
14.	G6PD V	0.00	0.00	0.00	0.00	0.00
15.	GDH I	0.67	0.48	0.92	0.32	0.20
16.	GDH II	0.00	0.00	0.00	0.00	0.00
17.	IDH I	0.00	0.00	0.00	0.00	0.00
18.	IDH II	0.00	0.15	0.00	0.20	1.00
19.	LDH I	0.00	0.00	0.00	0.00	0.00
20.	LDH II	0.00	0.00	0.05	0.68	0.00
21.	LDH III	1.00	0.50	0.00	0.63	1.00
22.	MDH I	0.00	0.00	0.35	0.00	0.50
23.	MDH II	0.00	0.00	0.00	0.00	0.00
24.	XDH I	0.16	0.91	0.58	0.58	0.63
25.	XDH II	0.00	0.00	0.00	0.00	0.00

6.3 AVERAGE NUMBER OF ALLELES AND PROPORTION OF POLYMORPHIC LOCI

In population genetic analysis informations on average number of alleles per loci and average number of polymorphic loci are also used in evaluating the genetic variability between the populations and finally within the species itself. The Table 7 and 8 show detailed informations on the number of loci tested and the number of alleles observed in populations of S. longiceps from Cochin, Calicut, Mangalore, Mandapam and Madras. The average number of alleles among these five populations varied from 1.28 in Cochin population to 1.49 in Mandapam population showing considerable differences existing in the nature of allelic distribution among these populations. The average number of alleles was also considerably different between populations from east and west coast. It varied from 1.28 to 1.40 between Cochin and Calicut populations on the west coast and from 1.29 to 1.49 between Madras and Mandapam populations from the east coast. Interestingly, the average number of alleles between Calicut and Mangalore was almost similar; it being 1.40 in Calicut and 1.39 in Mangalore. The average number of alleles in the species was thus found to be 1.37 which appears to be considerably high compared to many other fish species. However, screening of other tissues not tested in the present investigation can affect these values naturally. The present estimate of average number of alleles revealed good amount of genetic polymorphism in the species.

The average number of polymorphic loci estimated for different populations of S. longiceps is shown in the Table 7. It varied from 0.33 in Cochin

Table : 7

Comparison of Average Polymorphic Loci in

Sardinella longiceps

Sl. No.	Enzyme	No. of loci present					No. of Polymorphic loci					Average No. of Polymorphic loci				
		CHN	CCT	MRE	MDM	MAS	CHN	CCT	MRE	MDM	MAS	CHN	CCT	MRE	MDM	MAS
1.	ADH	2	2	2	2	2	0	2	0	2	0	0	1	0	1	0
2.	AO	3	3	3	3	3	2	1	1	1	1	0.60	0.30	0.30	0.30	0.30
3.	EST	4	4	4	4	4	4	4	4	3	3	1	1	1	0.75	0.75
4.	G6PD	5	5	5	5	5	0	0	0	1	1	0	0	0	0.20	0.20
5.	GDH	2	2	2	2	2	1	1	1	2	1	0.50	0.50	0.50	1	0.50
6.	IDH	2	2	2	2	2	0	1	0	1	0	0	0.50	0	0.50	0
7.	LDH	3	3	3	3	3	1	1	2	2	1	0.33	0.33	0.66	0.66	0.33
8.	MDH	2	2	2	2	2	0	0	1	0	1	0	0	0.50	0	0.50
9.	XDH	2	2	2	2	2	1	1	1	1	1	0.50	0.50	0.50	0.50	0.50
	Total	25	25	25	25	25	9	11	10	13	9	2.93	4.13	3.46	4.91	3.08
	Average No. of Polymorphic Loci in each population =											0.33	0.46	0.38	0.55	0.34

Average No. of Polymorphic Loci in Sardinella longiceps 0.41

Table : 8

Comparison of Average Number of Alleles at 25 Loci in *Sardinella longiceps* populations

Sl. No.	Enzyme	No.of Loci	COCHIN				CALICUT				MANGALORE				MANDAPAM				MADRAS			
			No. of alleles		Total alleles	Ave-rage	No. of alleles		Total alleles	Ave-rage	No. of alleles		Total alleles	Ave-rage	No. of alleles		Total alleles	Ave-rage	No.of alleles		Total alleles	Ave-rage
			S	F			S	F			S	F			S	F			S	F		
1.	ADH	2	2	0	2	1	2	2	4	2	2	0	2	1	2	2	4	2	2	0	2	1
2.	AO	3	3	2	5	1.66	2	2	4	1.33	2	2	4	1.33	3	1	4	1.33	3	1	4	1.33
3.	EST	4	4	4	8	2	4	4	8	2	4	4	8	2	4	3	7	1.75	4	3	7	1.75
4.	G6PD	5	5	0	5	1	5	0	5	1	5	0	5	1	5	1	6	1.2	5	1	6	1.2
5.	GDH	2	1	2	3	1.5	2	1	3	1.5	1	2	3	1.5	2	2	4	2	1	2	3	1.5
6.	IDH	2	1	1	2	1	2	1	3	1.5	1	1	2	1	2	1	3	1.5	1	1	2	1
7.	LDH	3	2	2	4	1.33	2	2	4	1.33	3	2	5	1.66	3	2	5	1.66	2	2	4	1.33
8.	MDH	2	1	0	1	0.5	1	0	1	0.5	2	1	3	1.5	1	0	1	0.5	1	1	2	1
9.	XDH	2	2	1	3	1.5	2	1	3	1.5	2	1	3	1.5	2	1	3	1.5	2	1	3	1.5
	Total	25			33	11.49			35	12.66			35	12.49			37	13.44			33	11.61
	Average No. of alleles in each population					1.28				1.41				1.39				1.49				1.29
	Average No. of alleles in *Sardinella longiceps*					1.37																

to 0.55 in Mandapam populations, again indicating considerable differences in the average number of polymorphic loci among populations of S. longiceps. It varied from 0.33 to 0.46 between populations from west coast and 0.34 to 0.55 between populations from the east coast indicating considerable differences between east and west populations. The present estimate of average number of polymorphic loci, 0.41 in S. longiceps also appear to be higher than that of many species reported in the literature. The proportion of polymorphic loci for fish in general has been reported as 0.31 by Selander (1976) as discussed by Shaklee (1984). Similarly Ayala and Kiger (1980) have reported a proportion of polymorphic loci of 0.306 for fourteen fish species.

Many investigators have reported high range of proportion of polymorphic loci and average number of alleles. For example, in mosquito fish populations, Hernandez-Martich and Smith (1990) have reported a percentage polymorphic loci of 47.6 and an average number of alleles as 1.56 in twenty one loci tested. Similarly Grant (1984) has also reported 48 per cent of polymorphic loci out of the fourty loci tested in his study in Atlantic herring, Clupea harengus populations. Okazaki (1986) has reported a fifty percent polymorphic loci out of thirty four loci tested. It may appear as a significantly higher value of genetic variability. Interestingly, a still higher value of proportion of polymorphic loci and average number of alleles was recently reported in Mugil cephalus (Vijayakumar, 1992). In M. cephalus populations, average number of polymorphic loci and average number of alleles varied from 0.61 to 0.71 and 1.72 to 1.82 respectively which again averaged 0.67 and 1.77. Highest percentage of polymorphic loci (0.72) was observed in

Cochin population whereas highest value of average number of alleles was observed (1.82) in Madras population. Similarly, lowest value (0.61) of proportion of polymorphic loci was observed in Madras population whereas lowest value of average number of alleles was observed in (1.72) Cochin population. Though a comparison of the present value of 0.41 proportion of polymorphic loci with that of Selander (1976) or that of Ayala and Kiger (1980) may appear as slightly higher, a comparison with other reports on high proportion of polymorphic loci can reveal that the present value is not unusual. The highest value of average number of alleles per polymorphic loci, 3.3 was reported in damsel fish, Stegastes fasciolatus (Shaklee, 1984).

Higher values of proportion of polymorphic loci and average number of alleles were also reported in shell fishes and molluscs. A typical example of such kind is that of De Matthaeis et al. (1983). They have reported 39% of polymorphic loci and 1.48 average number of alleles in the species. Penaeus japonicus. Similarly Fujio et al. (1983) have reported a high value of 0.41 as the average number of alleles in marine mollusc populations.

From the above discussions on varying values of proportion of polymorphic loci and average number of alleles, it is clear and certain that no comparable standard values can be fixed for a particular species or its populations in a particular region. Therefore, the values observed for proportion of polymorphic loci and average number of alleles in oil sardine, S. longiceps in the present investigation may be natural for the species. Also, the reason for low and high range of genetic variability between species

population and between localities cannot be justifiably correlated to any comparable phenomenon, because the genetic variability may depend on so many factors like environmental, geographic, migratory behaviours and certain other unknown factors. Thus the utility or the advantage of measuring these above discussed values such as Chi-square for genotype distribution, proportion of polymorphic loci, average number of alleles and heterozygosity etc. is that it contributes to the general efficiency and credibility of the methodology adopted, the data analysed and the very evaluation and conclusion made on the population genetics of S. longiceps studied here.

6.4 BANDING PATTERNS OF ENZYMES

In modern population biochemical genetic studies a knowledge on the subunit structure of enzyme/proteins also helps in the interpretation of electrophoretic phenotypes as genotypes. Because, depending on the structure of enzymes, the number of bands in the homozygotes and heterozygotes may vary. Thus in heterozygous condition, a monomer expresses two bands, dimer expresses three bands and tetramer expresses five bands (Shaw and Prasad, 1970).

ADH is reported to be a dimeric enzyme in structure (Harris and Hopkinson, 1978; Ward, 1978; Grant et al., 1987; Berg and Gall, 1988) showing three banded heterozygote and single banded homozygote. However, ADH-I locus in S. longiceps showed only two banded heterozygote structure, whereas three banded heterozygote structure was shown at ADH-II locus. Such deviations from the expected pattern of dimeric structure was also

reported in Tilapia zilli (Cruz et al., 1982). In Tilapia the missing band appeared to be the middle interlocus hybrid band.

AO in S. longiceps showed two banded heterozygote and a single banded homozygote. Similar observation was also reported in Penaeus indicus (Philip Samuel, 1987) and Mugil cephalus (Vijayakumar, 1992).

Esterase phenotype patterns observed in homozygous and heterozygous condition showed a monomeric structure. Homozygotes showed single banded pattern and heterozygotes showed double banded condition. Though double banded heterozygotes showed a monomeric structure in all the populations except Cochin population, where three banded heterozygotes were observed at third locus suggesting a dimeric structure. Monomeric esterase have been reported by many authors (Underhill, 1974; Johnson, 1975; Cruz et al., 1982; Sumantadinata and Taniguchi, 1982; Shaklee and Samollow, 1984; Crozier, 1987; Milton and Shaklee, 1987). However, dimeric enzyme structure has been reported in Mugil cephalus (Vijayakumar, 1992). He observed two bands for homozygotes and three bands for heterozygotes. Similar two banded homozygote and three banded as well as four banded heterozygotes were reported at EST locus 3 in the blue gill (Avise and Smith, 1974). Similarly, Ward (1978) reported a dimeric enzyme structure in mammals. Besides, in certain species esterase enzyme appeared to be monomeric in one tissue and dimeric in another (Salini and Shaklee, 1988). Thus the subunit structure of esterase appears to vary even within the species and between species as reported by Vijayakumar (1992). It is rather too difficult to explain the

present observation of dimeric structure for EST-III locus only in Cochin population. To know whether it was produced by some unknown artefact, repeated sampling and testing are necessary. Since the characterization of chemical structure of enzymes does not come under the purview of the present investigation, attempt has not been made to characterise the chemical structure.

In S. longiceps G6PD and GDH enzymes showed monomeric structure. However, G6PD was reported to be a dimer in mammals and a tetramer in fish and amphibians (Yamauchi and Goldberg, 1973; Hori et al., 1975). Cruz et al. (1982) observed two banded heterozygotes and therefore considered GDH as a monomer as in other vertebrates (Harris and Hopkinson, 1978).

IDH is a dimeric molecule in most animals including fishes (Johnson, 1975; Cruz et al., 1982; Berg and Gall, 1988). In the present investigation also IDH was considered as a dimer. However, only two banded phenotypes were observed since the heterodimer band of intermediate mobility was too weak to be resolved with the available technique.

As regard to LDH zymogram patterns, most of the specimens from Cochin and Madras showed five banded heterozygote patterns and the rest having less than five but more than three bands. This five banded structure is expected for a functionally tetrameric enzyme. Eventhough five banded tetrameric structure of LDH has been reported by various investigators (Johnson 1975; Cruz et al., 1982; Bartley et al., 1985; Grant et al., 1987; Berg and

Gall, 1988), there are reports on lesser number of LDH heterozygote bands. For example, Kornfield and Koehn (1975) observed usually only three and infrequently four bands in heterozygote condition in Cichlids. The reason attributed to this was restricted subunit assembly (Whitt and Horowitz, 1970). Smaller number of bands was commonly observed in fishes as reported by Kornfield and Koehn (1975). Markert and Faulhaber (1965) observed that five LDH isozymes were not always present in muscle extracts as reported by Clayton and Franzin (1970). The major reason for not observing the expected five banded pattern in the present case may be due to the condition of the tissue causing light band becoming less resolved and thus too light to be visible.

MDH and XDH in the present investigation showed two banded heterozygotes and single banded homozygotes. However, both enzymes have been reported as a dimer by some of the investigators. (Clayton et al., 1971; Johnson, 1975; Smith et al., 1978; Grant and Utter, 1980; Smith and Jamieson, 1980; Cruz et al., 1982; Fujio et al., 1983; Bartley et al., 1985; Berg and Gall, 1988). It may be that one of the three bands was too weak to resolve in the present experimental conditions followed as in the case of LDH. The above discussion shows clearly that lesser number of bands observed in some enzyme systems has not affected the interpretation of the results of the present investigation.

The major findings of the present investigation are high genetic variability within the species, S. longiceps and significant allele frequency

differences between its populations. It is important to ask whether the above two findings were influenced by differences in the sample size of populations used for comparison. The sample size in Cochin was comparatively larger than all other regions in most of the enzyme loci considered (TBL.3). From the statistical point of view, the chances of observing polymorphism if present, is comparatively higher in larger sample size. In this respect, it is interesting to point out that many loci at Cochin, where sample size was larger than that of other centres were not polymorphic whereas, high polymorphism was observed in other centres with only smaller sample size. For example enzyme loci ADH-I, II; G6PD-III; GDH-II; IDH-II; LDH-II and MDH-I (TBL.2). The above explained observation is also true with many other loci where higher rate of polymorphism occured in places where smaller sample size was tested. For example AO-II, III; EST-I-IV; GDH-I; LDH-III and XDH-I. Hence the observed results were least affected by the sample size. In fact the observed pattern of results as discussed above suggests a positive evidence for natural differences existing between these populations irrespective of their sample size.

6.5 GENETIC IDENTITY AND DISTANCE

Though a comparative analysis of allele frequencies at twenty five loci has already revealed genetic stock differences in S. longiceps, it may be interesting to measure the degree of such differences from the point of view of evolutionary period of such genetic differentiation taken place in these various populations. In this respect, a popular method applied for

such estimates is genetic identity and genetic distance between populations as suggested by Nei (1972). Genetic identity estimates the proportion of genes that are identical in structure in two populations, the value of which can vary from 0-1. The zero value for genetic identity indicates that no common alleles are shared between the populations sampled whereas latter value is indicative of homogeneous populations. The genetic distance estimates the number of allelic substitutions per locus that have occurred in the separate evolution of two populations (Ayala and Kiger, 1980). Here the genetic distance value can range from Zero to >1. Thus the values of genetic distance may be described as an indirect index of evolutionary distance moved away by the concerned populations (Vijayakumar, 1992).

The average values of genetic identity and genetic distance estimated for S. longiceps in the present investigation were 0.9065 (I) and 0.0990 (D) respectively. The estimate involved 250 pairwise comparison of five populations (Cochin, Calicut, Mangalore, Mandapam and Madras) with respect to twenty five enzyme loci examined (TBL.10). The average (I) and (D) values for interbreeding local fish populations may be 0.98 and 0.020 respectively (Ayala and Kiger, 1980). Therefore, the value of the interpopulation genetic identity and distance estimated here for S. longiceps are significantly different, the genetic identity being less (0.90) and genetic distance being higher (0.09) than the above mentioned expected values for interbreeding local populations. A comparative genetic identity and distance existing among five populations of S. longiceps tested in the present investigation may be

Table 10

Paired comparison of values of Genetic Identity (I) and Genetic Distance (D) between Cochin, Calicut, Mangalore, Mandapam and Madras populations of *Sardinella longiceps*

Sl. No.	Locus		CHN/CCT	CHN/MRE	CHN/MDM	CHN/MAS	CCT/MRE	CCT/MDM	CCT/MAS	MRE/MDM	MRE/MAS	MDM/MAS
1.	ADH	I	0.9487	1.0000	0.9363	1.0000	0.9487	0.9993	0.9487	0.9363	1.0000	0.9363
2.	ADH	II	0.9986	1.0000	0.9487	1.0000	0.9986	0.9640	0.9986	0.9487	1.0000	0.9487
3.	AO	I	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
4.	AO	II	0.9363	0.9363	0.7098	0.9796	1.0000	0.9119	0.9878	0.9119	0.9878	0.8368
5.	AO	III	0.9744	0.9744	0.2249	0.2249	1.0000	0.0000	0.0000	0.0000	0.0000	1.0000
6.	EST	I	0.9989	0.9718	0.9520	0.9520	0.9595	0.9363	0.9363	0.9973	0.9973	1.0000
7.	EST	II	0.8321	0.8262	0.9806	0.8944	0.3750	0.7071	0.4962	0.9207	0.9909	0.9648
8.	EST	III	0.9326	0.9171	0.9602	0.9003	0.9992	0.7946	0.9967	0.7692	0.9992	0.7428
9.	EST	IV	0.9998	0.9036	0.9902	0.9795	0.8942	0.9930	0.9749	0.8349	0.9714	0.9417
10.	G6PD	I	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
11.	G6PD	II	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
12.	G6PD	III	1.0000	1.0000	0.2942	0.6635	1.0000	0.2942	0.6635	0.2942	0.6635	0.9103
13.	G6PD	IV	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
14.	G6PD	V	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
15.	GDH	I	0.7277	0.9817	0.9476	0.9211	0.8450	0.4705	0.4033	0.8694	0.8301	0.9972
16.	GDH	II	0.0000	1.0000	0.4191	1.0000	0.0000	0.9080	0.0000	0.4191	1.0000	0.4191
17.	IDH	I	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
18.	IDH	II	0.9953	1.0000	0.5011	1.0000	0.9953	0.5826	0.9953	0.5011	1.0000	0.5011
19.	LDH	I	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
20.	LDH	II	1.0000	0.9767	0.8918	1.0000	0.9767	0.8918	1.0000	0.9682	0.9767	0.8918
21.	LDH	III	1.0000	0.9608	0.9434	1.0000	0.9608	0.9434	1.0000	1.0000	0.9608	0.9434
22.	MDH	I	1.0000	0.7926	1.0000	0.9191	0.7926	1.0000	0.9191	0.7926	0.9687	0.9191
23.	MDH	II	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
24.	XDH	I	0.8188	0.9543	0.9543	0.9410	0.9530	0.9530	0.9648	1.0000	0.9991	0.9991
25.	XDH	II	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
	GI		0.9265	0.9678	0.8661	0.9351	0.9079	0.8539	0.9092	0.8465	0.9338	0.9180
	GD		0.0763	0.0327	0.1438	0.0671	0.0966	0.1579	0.0952	0.1666	0.0685	0.0856

Average genetic identity in *Sardinella longiceps* = 0.9065

Average genetic distance in *Sardinella longiceps* = 0.0990

evaluated from the range of values obtained, 0.846-0.967 (I) and 0.033 0.166 (D) (TBL.10). In terms of these values, the populations drawn from Mangalore and Mandapam with 0.846 genetic identity and 0.166 genetic distance appeared to be the most distant or heterogeneous groups followed by Calicut-Mandapam and Cochin-Mandapam. These higher order of genetic distances are interestingly correlated to their comparable geographical distances. The highest genetic distance (D) being indicated between Mangalore population in the west coast and Mandapam population in the east coast of India. This apparent order of higher correlation between distant populations continued between Calicut-Mandapam and Cochin-Mandapam but at lesser level between Calicut-Mangalore, Cochin-Calicut and Cochin-Mangalore (TBL.10). The average genetic identity and distance values of three west coast populations could be calculated as 0.95 (I) and 0.065 (D) and between west coast and east coast could be calculated as 0.90 (I) and 0.1165 (D) from the Table 10. These values suggest that all the west coast populations have more genetic similarity or less genetic distance than that of east coast. On the other hand, these two values between Mandapam and Madras populations, 0.918 (I) and 0.0856 (D), reveal that these two local populations on the east coast are genetically more dissimilar or distant than west coast populations themselves. Thus the geographical locations might have influenced in the degree of diversification of these west and east coast populations. However, more explanations are needed for the high degree of genetic differentiation between Mandapam and Madras populations. Besides, the parental stocks of west and east coast oil sardine fishery may be different. Because,

the history of east coast oil sardine fishery appears to be of recent origin (Devanesan and Chidambaram, 1953; Anon, 1989a,b) and whereas it is known to form a fishery in Sri Lanka and Andaman sea (Bal and Rao, 1984). Hence it is advisable to compare genetic composition of oil sardine populations of these regions. The significant genetic heterogeneity between the closely existing Calicut-Mangalore population also appear to be real because significant differences in the allelic frequencies at several enzyme loci were also noticed between these two populations as discussed elsewhere.

Reports on higher D values between fish/shell fish populations are also not uncommon (Stoneking et al., 1981; Liu et al., 1991; Vijayakumar, 1992). A typical example is that of Liu et al. (1991). In their study in Stramonita haemostoma, a higher value of 0.30 (D) was recorded between two groups of the same species. Based on this D value the two groups were differentiated genetically at a level that is characteristic of congeneric species. However, Stoneking et al. (1981) observed D value of 0.116 between northern and southern populations of brook trout and considered them at the level of sub species differences. Similarly a relatively low average genetic similarity coefficient (D=0.16) among populations of Campostoma anomalum have been reported by Buth and Burr (1978). The range of D values and their average in different marine and fresh water fish species have been reviewed by Shaklee et al. (1982). These values averaged like 0.05 (range 0.002-0.065) between conspecific populations, 0.30 (0.025-0.609) between related species and 0.90 (0.580-1.21) between related genera. Similarly, Mangaly and Jamieson (1978) also reported that values for Nei's coefficient of genetic

distance may range upto about 0.05 between races and from 0.02 to 0.20 between sub-species. However, interlocus variance of genetic distance may be very high (Nei, 1976). Therefore based on the above discussion the average genetic identity value of 0.09 in S. longiceps in the present investigation is well between the average for conspecific populations and subspecies. As genetic identity values are complementary to the genetic distance values, only the latter is discussed in detail.

Interestingly, in contrary to the above discussed reports on range and their average of D values, many authors have found very small D values also between different species and even between different genera (Ryman et al., 1979; Smith et al., 1980; Grant, 1986). A typical example in this regard is that of Smith et al. (1980). A genetic distance of only 0.003 was found between two species and they suggested that they are conspecific populations. Most unusual range of genetic distance was reported in Atlantic herring populations (Jorstad et al., 1991). They observed a range of 0.005-0.010 to 0.888-1.086. The D values 0.888 to 1.086 were for two adjacent populations. Another atypical range of genetic distance values of 0.092 to 0.204 with an average of 0.152 was reported in mosquito fish populations (Hernandez-Martich and Smith, 1990). Vijayakumar (1992) also has reported D value of 0.152 in Mugil cephalus populations and has suggested that the populations of M. cephalus have diverged into a level of genetic distance lying between races and sub-species.

In addition to the above reports of unusual genetic distance between populations of same species, sub-species, species etc., some investigators

have reported very small D values between widely separated populations. For example, in Chanos chanos populations, the average genetic distance observed was only 0.002 (Winans, 1980). It is interesting to note that small D values were observed for different populations which were separated by large distance, even upto 10,000 kilometers range, whereas significant genetic divergence was noticed for other populations separated by just 320 kilometers only. However, the adjacent populations were isolated by local oceanic conditions. Another interesting report on small D value is that of Ryman et al. (1979). They observed reproductive isolation with little genetic divergence (D=0.025) in populations of brown trout. Thus from the above discussed examples, long distance between populations need not necessarily reflect a high genetic divergence, because isolation by any form of known or unknown barrier between two populations, rather than the long distance, appears to be the major cause for high genetic divergence. A typical example of such kind is the range of genetic distance values viz. 0.13-0.36 reported for shore fishes separated by Isthumus of Panama, though some of which showed morphological similarity (Gorman et al., 1976; Gorman and Kim, 1977; Vawter et al., 1980) as discussed by Rosenblatt and Waples (1986).

Again, the above detailed discussion on reports of values of genetic distance in various species and in various geographical situations suggests a very high range of values even between two closely existing populations and least range of values between populations inhabiting extreme geographical range and also a medium range significant values between populations of

a particular species and in a particular situation all of which suggest that no standard parameter of genetic identity and distance can be recommended for any species. Therefore, a satisfactory approach for concluding on the genetic nature of a particular species could be combined differences estimated at various levels of population genetic analysis such as allelic frequencies, proportion of polymorphic loci, average heterozygosity and values of genetic identity (I) and genetic distance (D). When all these different levels of genetic analyses suggest a uniform conclusion which may be more valid for the species in question than comparison of conclusions made by other investigators for other species. Though values of genetic distance for S. longiceps are higher than expected between local populations but lesser than that expected for sub-species, it may be too early to assign any genetic status to these populations without further required investigations.

The earlier studies of various aspects of fishery and biology of S. longiceps have suspected heterogenetic nature of its populations supporting the fishery (Anon, 1990). The studies on vertebral counts of different oil sardine populations along the North Kanara coast clearly indicated the existence of meristically distinct stocks. In this respect, the more interesting informations contained in the above report are that samples of juveniles (below 160 mm) and adults (above 160 mm, mainly spent - recovering ones) tested from each centre were homogeneous, while pooling of these data caused significant statistical differences, indicating heterogeneity of different populations (Annigeri, 1978). It also suggested that these local sub-populations

within a range of about 40 kilometres may have their own separate spawning grounds and enter any local fishing grounds for feeding purpose. Similarly based on a comparative study of head and tail lengths of oil sardine samples of various geographical areas, including Bombay and Malabar, Devanesan and Chidambaram (1943) also suspected the existence of different races among oil sardine populations. Devanesan (1943) also reconfirmed considerable head-length differences between Malabar and Bombay-Karachi region. Though oil sardine, S. longiceps is believed to be a pelagic migratory fish, its movements for feeding and spawning are appeared to be restricted from local feeding grounds to the spawning grounds and vice versa (Devanesan, 1943). It is also interesting to learn that there is considerable difference in the feeding habit of oil sardine between Bombay and Malabar region as reported by Devanesan (1943).

Various studies on size groups of oil sardine from different places such as Cochin (Balan, 1972; Reghu, 1973), Calicut (Rengaswamy, 1977), Mangalore (Prabhu and Dhulkhed, 1967), Kasargod (Anon, 1974), Kakinada (Antony Raja, 1973a) and Parangipettai (Kumar and Balasubramaniam, 1987) also revealed regional differences in the year class sizes which may indirectly support the existence of heterogeneous oil sardine populations along west-east coast. Besides, based on the observations on significant regional size-group differences, Antony Raja (1969) suspected the existence of two sub populations at Mangalore and Calicut.

A number of reports on spawning and related aspects of oil sardine in fact agree that spawning occurs independently at Mangalore/Karwar (Prabhu

and Dhulkhed, 1967; Annigeri, 1969; George, 1980), Calicut (Velappan Nair, 1959; Rengaswamy, 1977), Cochin (Balan, 1972; Reghu, 1973; Balan and Abdul Nizar, 1988), Vizhinjam (Lazarus, 1985) and east coast regions such as Madras, Gulf of Mannar (John, 1951; Gnanamuthu and Giridavallabhan, 1984). Kesavan Nair et al. (1973) also suspected patchy distribution of oil sardine populations suggesting some form of isolation between populations.

In the above background of earlier reports, the present discovery of the existence of distinct genetic stocks of oil sardine at Cochin, Calicut, Mangalore, Mandapam and Madras though not surprising, it is of more scientific importance. As genetic method was used in the present investigation, the present report of existence of distinct genetic stocks of oil sardine along the west and east coast of India must be more natural and reliable. If these tested samples on the east and west coast are distinct genetic stocks, separate spawning grounds for each such stock may exist as suspected by Annigeri (1978) for sub populations along the North Kanara coast. Finally, the recent investigation on 25 morphometric characteristics of oil sardine populations from Mangalore, Calicut, Cochin and Mandapam also revealed that all these four populations belonged to significantly different stocks (Personal Communication from Mohan Das, 1993). Therefore, all these discussions made on various aspects of fishery and biology of oil sardine lent strong support to the present report on the existence of genetically distinct stocks of oil sardine in Cochin, Calicut, Mangalore, Mandapam and Madras.

7. CONCLUSIONS

1. The average values of proportions of polymorphic loci (0.41), alleles per locus (1.37) and heterozygosity (0.21) estimated for Sardinella longiceps revealed high genetic variability in the species.

2. Significant allelic frequency differences at 14 out of 25 enzyme loci suggest that the populations of S. longiceps tested from Cochin, Calicut, Mangalore, Mandapam and Madras are genetically distinct stocks (TBL.2). Allelic frequency differences at a single enzyme system like esterase (EST) or glutamate dehydrogenase (GDH) were sufficient to reveal inherent stock differences of all the five populations compared.

3. The degree of population genetic differentiation estimated by mean values of genetic identity (I=0.9065) and Distance (D=0.0990) also reveals that the populations tested are not genetically identical. The lowest genetic identity (0.846) or the corresponding highest distance (0.166) exists between populations of Mangalore and Mandapam regions (TBL.10).

8. RECOMMENDATIONS

1. As oil sardine fishery is supported by genetically heterogeneous populations, independent data for each distinct stock must be taken into account for successful forecast on its abundance as well as management of oil sardine fishery resources of India.

2. The present findings will have very significant implications on the scientific management of oil sardine fishery resources in the future. Therefore, it is recommended that mitochondrial DNA patterns of different populations of oil sardine should be compared to get an insight into the subtle nature of their genetic composition.

3. The present investigation does not give any clue to the natural spawning boundaries of each of these distinct genetic stocks and their mixing during feeding season. Therefore, it is necessary to study separately the genetic composition of the regional populations of oil sardine at the time of spawning as well as feeding seasons.

9. SUMMARY

1. The thesis contains a detailed account of the results of the investigation on the BIOCHEMICAL GENETIC STUDIES ON THE POPULATIONS OF THE INDIAN OIL SARDINE, SARDINELLA LONGICEPS from Cochin, Calicut, Mangalore of west coast and Mandapam, Madras of east coast of India. Introduction, Review of Literature, Materials and Methods, Results, Discussion, General Discussion, Conclusions, Recommendations, Summary and References are the major headings of the thesis.

2. A brief account on the concept of species, speciation, importance of species concept in fisheries management, definitions of units of fisheries management, role of biochemical genetic techniques in the identification of unit stock, reasons for choice of S. longiceps as the candidate for the investigation and the major objectives of the investigation itself is given in the Introduction.

3. The Review of Literature has covered the available published reports and informations with special reference to population biochemical genetics using different methods.

4. Under Materials and Methods, details on oil sardine population samples, methods of transportation of samples, preparation of tissue homogenate and buffer systems for gel electrophoresis, methods of staining and detection of 9 enzyme systems, procedures for analysis of data etc. have been presented.

5. The results have been described with the help of 19 plates, 19 figures and 10 tables. The fine aspects of zymogram patterns observed at

25 loci belonging to 9 enzyme systems (ADH, AO, EST, G-6-PD, GDH, IDH, LDH, MDH and XDH) present in oil sardine populations tested from Cochin, Calicut, Mangalore, Mandapam and Madras have been highlighted. The important biochemical genetic characteristic of oil sardine populations discovered for the first time are:

a. Intra species biochemical genetic polymorphism measured by proportion of polymorphic loci ranging from 0.33 in Cochin to 0.55 in Mandapam, giving an average of 0.41 in the species (TBL.7).

b. The average heterozygosity also varied from 0.16 in Mangalore to 0.24 in Mandapam giving an average of 0.21 in the species (TBL.5).

c. The average number of alleles per locus varied from 1.28 in Cochin to 1.49 in Mandapam, giving a species average of 1.37 (TBL.8). All these three characteristics suggest high genetic variability in the species.

d. The pevotal data required for achieving the main objective of the investigation emerged from the results on allelic frequencies estimated for 25 individual enzyme loci (TBL.2). The loci that showed very significant allelic frequency differences between populations are: ADH-I, II; AO-II, III; EST-I-IV; G-6-PD-III; GDH-I, II; IDH-II; MDH-I and XDH-I. Besides, allelic frequency differences at a single enzyme system like esterase (EST) or glutamate dehydrogenase (GDH) were sufficient to reveal inherent stock differences of all the 5 populations compared (TBL.2).

e. Significant deviations between observed and expected genotype frequencies (TBL.3) occurred only in 24 cases out of 125 compared and these deviations were caused by excess of heterozygotes in 17 occasions and the rest 7 cases due to excess of homozygotes (TBL.6).

f. The estimated overall mean values of genetic Identity (I) and distance (D) ranged from 0.846 to 0.967 (I) and 0.033 to 0.166 (D) respectively. The mean values for the species were 0.9065 (I) and 0.0990 (D), suggesting that the populations compared are less identical and more distant than expected between interbreeding local populations (TBL.10).

6. All the salient features of the present investigation have been thoroughly discussed under two headings namely DISCUSSION and GENERAL DISCUSSION. In the first part of discussion, important background informations essentially required for proper and meaningful evaluation of the results were introduced. The second part of it comprised of a detailed discussion on the results obtained for each of the 9 enzyme systems, highlighting the allelic frequency differences at interpopulation level, viz., 14 out of the 25 enzyme loci examined showed very significant allelic frequency differences in the oil sardine populations from Cochin, Calicut, Mangalore, Mandapam and Madras. It was concluded that these 5 populations were genetically distinct stocks

(TBL.2). The conclusions reached under each enzyme system were supported by similar reports in other species.

7. The chapter, General Discussion, first described the role of Hardy-Weinberg law in population genetic analysis and the results of its application in the present investigation. Relatively small number of significant deviations, 24 out of 125 tested, were justified as normal events that can occur in any similar investigations as supported by references. Possible reasons for such deviations have also been discussed. The next aspect discussed in this section was the very high genetic variability expressed by average heterozygosity ranging from 0.16 in Mangalore to 0.24 in Mandapam. Possible reasons for high heterozygosity values in S. longiceps have also been suggested. Other aspects discussed in this section are, high rate of average number of alleles per locus ranging from 1.28 in Cochin to 1.49 in Mandapam and the proportion of polymorphic loci ranging from 0.33 in Cochin to 0.55 in Mandapam and probable structure of the enzymes tested in the present investigation. An important aspect that was discussed in the section was, nature of the genetic identity and distance that existed between populations of S. longiceps compared in the present investigation. The average values 0.9065 for genetic identity (I) and 0.0990 for genetic distance (D) suggest that five populations of S. longiceps compared are not genetically identical. The lowest genetic identity (0.846) or the corresponding highest genetic distance (0.166) occurred between Mangalore and Mandapam populations, the two almost farthest geographic regions among the areas compared (TBL.10 and

FIG.1). The final important informations discussed were the reports of earlier workers on the fishery and biology of oil sardine populations. The heterogeneity among oil sardine populations suspected by earlier workers has also been utilised as further evidence for the present conclusion that oil sardine populations from Cochin, Calicut, Mangalore, Mandapam and Madras are genetically heterogenous stocks.

8. Three important conclusions were drawn on the basis of the present investigation.

9. Three recommendations were also made for future lines of research.

10. REFERENCES

Allendorf, F.W., N. Ryman, A. Stennek and G. Stahl, 1976. Genetic variation in Scandinavian brown trout (Salmo trutta L.): evidence of distinct sympatric populations. Hereditas, **83(1):** 73-82.

Allendorf, F.W. and F.M. Utter, 1979. Population genetics. p. 407-454, In: W.S. Hoar and D.J. Randall (Eds.). Fish Physiology, Vol.8. Academic Press, New York.

Allendorf, F.W. and S.R. Phelps, 1981. Isozymes and the preservation of genetic variation in salmonid fishes. In: N. Ryman (Ed.). Fish Gene Pools. Ecol. Bull., (Stockholm) **34:** 37-52.

Allendorf, F.W., N. Ryman and F.M. Utter, 1987. Genetics and fishery management. In: N. Ryman and F.M. Utter (Eds.). Population Genetics and Fishery Management. Washington Sea Grant Program, University of Washington Press, Seattle, WA, pp. 1-19.

Altukhov, Yu. P., 1981. The stock concept from the viewpoint of population genetics. Can. J. Fish. Aquat. Sci., **38:** 1523-1538.

Altukhov, Yu. P. and E.A. Salmenkova, 1981. Applications of the stock concept to fish populations in the USSR. Can. J. Fish. Aquat. Sci., **38:** 1591-1600.

Andersson, L., N. Ryman and G. Stahl, 1983. Protein loci in Arctic charr, Salvelinus alpinus L. : electrophoretic expression and genetic variability patterns. J. Fish. Biol., **23:** 75-94.

Annigeri, G.G., 1969. Fishery and biology of the oil sardine at Karwar. Indian J. Fish., **16(1&2):** 35-50.

Annigeri, G.G., 1978. Studies on variations in the vertebral counts of oil sardine along the North Kanara Coast. Indian J. Fish., **25(1&2):** 14-22.

Anon, 1960. Proc. Joint. Scient. Meeting of ICNAF, ICES and FAO on "Fishing effort, the effect of fishing on resources and the selectivity of fishing". Lisbon. 27 May - 3 June 1957. Spec. Publs. Int. Commn. N.W. Atlant. Fish., **1(2):** 45 pp.

Anon, 1974. Plankton fish eggs and larvae studies. UNDP/FAO Pelagic Fishery Project IND/593 Progress Report, No.7. 52 pp.

Anon, 1981. Stock concept international symposium. Can. J. Fish. Aquat. Sci. (Special issue), **38(12):** 1457-1923.

Anon, 1989a. Marine fish production in India-1985-'86. Mar. Fish. Infor. Serv., T&E Ser., **91:** 1-32.

Anon, 1989b. On the emergence of oil sardine fishery along Madras coast. Mar. Fish. Infor. Serv., T&E Ser., **96:** 6-7.

Anon, 1990. The Indian oil sardine, Sardinella longiceps Valenciennes - An annotated bibliography. CMFRI Special Publication, No.48.

Antony Raja, B.T., 1969. The Indian oil sardine. Bull. Cent. Mar. Fish. Res. Inst. No.16, 128 pp. (Mimeograph).

Antony Raja, B.T., 1973. The Indian oil sardine fishery: problems in perspective. J. Mar. Biol. Assoc. India, **15(2):** 735-749.

Antony Raja, B.T., 1973a. Oil sardine in the estuaries of North Kanara. Indian J. Fish., **20(2):** 651-653.

Aspinwall, N., 1973. Inheritance of alpha-glycerophosphate dehydrogenase in the pink salmon, Oncorhynchus gorbuscha (Walbaum), Genetics, **73:** 639-643.

Avise, J.C. and M.H. Smith, 1974. Biochemical genetics of sunfish. I. Geographic variation and subspecific intergradation in the bluegill, Lepomis macrochirus. Evolution, **28:** 42-56.

***Ayala, F.J., M.L. Tracey, L.G. Barr, J.F. McDonald and S. Perez-Solar,** 1974. Genetic variation in natural population of five Drosophila species and the hypothesis of the selective neutrality of protein polymorphism. Genetics, **77:** 343-384.

Ayala, F.J. and J.A. Kiger, Jr., 1980. In: Modern Genetics. The Benjamin Cummings Publishing Company, Inc. 2727 Sand Hill Road, Menlo Park, California - 94025. 844 pp.

Bailey, G.S., H. Tsuyuki and A.C. Wilson, 1976. The number of genes for lactate dehydrogenase in salmonid fishes. J. Fish. Res. Bd. Canada, **33:** 760-767.

Bal, D.V. and K.V. Rao, 1984. The Oil Sardine. In: Marine Fisheries. Tata Mc Graw Hill Publishing Company Ltd., New Delhi. Chap. 4, p. 51-53.

Balan, V., 1972. Fishery and biology of the oil sardine, Sardinella longiceps Val., off the Cochin coast. Indian J. Fish., **18(1&2):** 135-147.

Balan, V. and M. Abdul Nizar, 1988. Purse seine fishery for oil sardine off Cochin during the year 1980-85. Indian J. Fish., **35(3):** 131-139.

Bartley, D.M., G.A.E. Gall and B. Bentley, 1985. Preliminary description of the genetic structure of white sturgeon, Acipenser transmontanus in the Pacific Northwest. In: F.P. Binkowski and S.I. Doroshov (Eds.). North American Sturgeons, 105-109, Dr. W. Junk Publishers, Dordrecht.

Basiao, Z.U. and N. Taniguchi, 1984. An investigation of enzyme and other protein polymorphisms in Japanese stocks of the tilapia Oreochromis niloticus and Tilapia zillii. Aquaculture, **38:** 335-345.

Benson, P.G. and P.J. Smith, 1989. A manual of techniques for electrophoretic analysis of fish and shell fish tissues. N.Z. Fish. Tech. Rep. No.13.

Berg, W.J. and G.A.E. Gall, 1988. Gene flow and genetic differentiation among California coastal rainbow trout populations. Can. J. Fish. Aquat. Sci., **45:** 122-131.

Berglund, A. and U. Lagercrantz, 1983. Genetic differentiation in populations of two Palaemon prawn species at the Atlantic east coast: does gene flow prevent local adaptation ? Mar. Biol., **77:** 49-57.

Bhattacharya, A. and J.R.B. Alfred, 1982. Electrophoretic variation of soluble protein fractions in tissues of different size groups of Channa stewartii and Danio dangila. Matsya, **8:** 23-32.

Bhosle, N.B., 1977. Intraspecific variation in the soluble nuclear eye lens proteins of the mullet, Mugil cephalus (L.). Mahasagar, **10(1&2):** 23-33.

Bock, W.J., 1980. The definition and recognition of biological adaptation. Am. Zool., **20:** 217-227.

Booke, H.E., 1968. Cytotaxonomic studies of the coregonine fishes of the Great Lakes, USA: DNA and Karyotype analysis. J. Fish. Res. Bd. Canada, **25:** 1667-1687.

Booke, H.E., 1981. The conundrum of the stock concept - are nature and nurture definable in fisheries science ? Can. J. Fish. Aquat. Sci., **38:** 1479-1480.

Boothroyd, E.R., 1959. Chromosome studies on three Canadian populations of Atlantic salmon, Salmo salar L. Can. J. Genet. Cytol., **1:** 161-172.

Brewer, G.J., 1970. In: Introduction to Izozyme Techniques. Academic Press, New York and London 186 pp.

Brown, L.D., 1991. Genetic variation and population structure in the blacklip abalone, Haliotis rubra. Aust. J. Mar. Freshwater Res., **42:** 77-90.

Busack, C.A., G.H. Thorgaard, M.P. Bannon and G.A.E. Gall, 1980. An electrophoretic, karyotypic and meristic characterization of the Eagle Lake trout, Salmo gairdneri aquilarum. Copeia, **3:** 418-424.

Buth, D.G. and B.M. Burr, 1978. Isozyme variability in the cyprinid genus Campostoma. Copeia, **2:** 298-311.

Buth, D.G., 1980. Evolutionary genetics and systematic relationships in the catostomid genus, Hypentelium. Copeia, **2:** 280-290.

Buth, D.G. and C.B. Crabtree, 1982. Genetic variability and population structure of Catostomus santaanae in the Santa Clara Drainage. Copeia, **2:** 439-444.

Bye, V.J. and A.G. Ponniah, 1983. In: Application of Genetics in Aquaculture. CMFRI Spl. Publ., No.13, 90 pp.

Campton, D.E. and F.M. Utter, 1987. Genetic structure of anadromous cutthroat trout (Salmo clarki clarki) populations in the Puget Sound area: evidence for restricted gene flow. Can. J. Fish. Aquat. Sci., **44:** 573-582.

Carl, L.M. and M.C. Healey, 1984. Differences in enzyme frequency and body morphology among three juvenile life history types of chinook salmon (Oncorhyncus tshawytscha) in the Nanaimo River, British Columbia. Can. J. Fish Aquat. Sci., **41:** 1070-1077.

Carlson, D.M., M.K. Kettler, S.E. Fisher and G.S. Whitt, 1982. Low genetic variability in paddle fish populations. Copeia, **3:** 721-725.

Casselman, J.M., J.J. Collins, E.J. Crossman, P.E. Ihssen and G.R. Spangler, 1981. Lake whitefish (Coregonus clupeaformis) stocks of the Ontario waters of Lake Huron. Can. J. Fish. Aquat. Sci., **38:** 1772-1789.

Christiansen, F.B. and O. Frydenberg, 1974. Geographical patterns of four polymorphisms in Zoarces viviparus as evidence of selection. Genetics, **77:** 765-770.

Clayton, J.W. and W.G. Franzin, 1970. Genetics of multiple lactate dehydrogenase isozymes in muscle tissue of lake white fish (Coregonus clupeaformis). J. Fish. Res. Bd. Canada, **27:** 1115-1121.

Clayton, J.W., D.N. Tretiak and A.H. Kooyman, 1971. Genetics of multiple malate dehydrogenase isozymes in skeletal muscle of wall eye (Stizostedion vitreum vitreum). J. Fish. Res. Bd. Canada, **28:** 1005-1008.

Clayton, J.W., 1981. The stock concept and the uncoupling of organismal and molecular evolution. Can. J. Fish. Aquat. Sci., **38:** 1515-1522.

Crozier, W.W., 1987. Biochemical genetic variation and population structure in angler fish Lophius piscatorius L. from the Irish sea and west of Scotland. J. Exp. Mar. Biol. Ecol., **106:** 125-136.

Crozier, W.W. and I.J.J. Moffett, 1990. Inheritance of allozymes in Atlantic salmon (Salmo salar L.). Aquaculture, **88:** 253-262.

Cruz, T.A., J.P. Thorpe and R.S.V. Pullin, 1982. Enzyme electrophoresis in Tilapia zilli: a pattern for determining biochemical genetic markers for use in tilapia stock identification. Aquaculture, **29:** 311-329.

Currens, K.P., C.B. Schreck and H.W. Li, 1990. Allozyme and morphological divergence of rainbow trout (Oncorhynchus mykiss) above and below waterfalls in the Deschutes River, Oregon. Copeia, **3:** 730-746.

* **Cushing, J.E.,** 1964. The blood groups of marine mammals. Adv. Mar. Biol., **2:** 85-131.

Daly, J.C. and B.J. Richardson, 1980. Allozyme variations between populations of baitfish species, Stolephorus heterolobus and St. devisi (Pisces: Engraulidae) and Spratelloides gracilis (Pisces: Dussumieriidae) from Papua New Guinea waters. Aust. J. Mar. Freshwater Res.. **31:** 701-711.

Das, P. and A.G. Jhingran (Eds.), 1989. In: Fish Genetics in India. Proceedings of the Symposium on Conservation and Management of Fish Genetic Resources of India, held on 11-13 April, 1986. Today and Tomorrow's Printers and Publishers, 24B/5 Deshbandhu Gupta Road, Karol Bagh, New Delhi. 266 pp.

Das, P., P.C. Mahanta and D. Kapoor, 1989. Conservation of marine fish genetic resources - present status and action needed. In: K. Algarswami, K. Alagaraja, M.S. Muthu, K.J. Mathew and N. Gopinatha Menon (Eds.). National Symposium on Research and Development in Marine Fisheries. CMFRI Bulletin No.44, pp. 159-162.

Davis, B.J., 1964. Disc electrophoresis II. Methods and application to human serum proteins. Annals. New York. Acad Sci., **121:** 404-429.

Dehring, T.R., A.F. Brown, C.H. Daugherty and S.R. Phelps, 1981. Survey of the genetic variation among eastern Lake Superior lake trout (Salvelinus namaycush). Can. J. Fish. Aquat. Sci., **38:** 1738-1746.

De Matthaeis, E., G. Allegrucci, A. Cocoone, D. Cesaroni, M. Cobabli Sbordoni and V. Sbordoni, 1983. Genetic differentiation between Penaeus kerathurus and P. japonicus (Crustacea, Decapoda). Mar. Ecol. Prog. Ser., **12:** 191-197.

Devanesan, D.W., 1943. A brief investigation into the causes of the fluctuation of the annual fishery of the oil sardine of Malabar, Sardinella longiceps: determination of its age and an account of the discovery of its eggs and spawning ground. Madras Fish. Bull., **28** (Report No.1): 1-24.

Devanesan, D.W. and K. Chidambaram, 1943. On the alleged existence of races in the population of oil sardines, Sardinella longiceps in the Arabian Sea. Proc. 30th Indian Sci. Congr., Calcutta (Abstract).

Devanesan, D.W. and K. Chidambaram, 1953. The Oil Sardine. In: The common food fishes of the Madras State. Government Press, Madras, p. 14-17.

Dhulkhed, M.H. and S.N. Rao, 1976. Electrophoretic studies on serum proteins of oil sardine (Sardinella longiceps) and mackerel (Rastrelliger kanagurta). Fish. Technol., **13(1):** 16-19.

Dietz, A.A., T. Lubrano and H.M. Rubinstein, 1972. In: Standard Methods in Clinical Chemistry, Academic Press, London. **1:** 323 pp.

Dobzhansky, Th., 1967. Evolution, genetics and man. Science Editions. John Wiley and Sons, Inc. New York.

Dobzhansky, Th., 1970. Genetics of the Evolutionary Process. Columbia University Press, New York and London.

Eckroat, L.R. and J.E. Wright, 1969. Genetic analysis of soluble lens protein polymorphism in brook trout, Salvelinus fontinalis. Copeia, **3:** 466-473.

Fevolden, S.E. and T. Haug, 1988. Genetic population structure of Atlantic halibut, Hippoglossus hippoglossus. Can. J. Fish. Aquat. Sci., **45:** 2-7.

Fujio, Y., R. Yamanaka and P.J. Smith, 1983. Genetic variation in marine molluscs. Bull. Japan Soc. Sci. Fish., **49(12):** 1809-1817.

Fuller, B. and L.J. Lester, 1980. Correlations of allozymic variation with habitat parameters using the grass shrimp, Palaemonetes pugio. Evolution, **34:** 1099-1104.

George, K.C., 1980. Studies on the distribution and abundance of fish eggs and larvae off the south-west coast of India with special reference to scombroids. Ph.D. Thesis, Cochin University of Science and Technology, Cochin, Kerala, India.

Gharrett, A.J., S.M. Shirley and G.R. Tromble, 1987. Genetic relationships among populations of Alaskan chinook salmon (Oncorhynchus tshawytcha). Can. J. Fish. Aquat. Sci., **44:** 765-774.

Gnanamuthu, J.C. and K.G. Girijavallabhan, 1984. A note on the occurrence of mature oil sardine, Sardinella longiceps Val., off Madras coast. Indian J. Fish., **31(3):** 378-379.

Gooch, J.L. and T.J.M. Schopf, 1971. Genetic variation in the marine ectoproct, Schizoporella errata. Biol. Bull., **141:** 235-246.

* **Gorman, G.C., Y.J. Kim and R. Rubinoff,** 1976. Genetic relationships of three species of Bathygobius from the Atlantic and Pacific sides of Panama. Copeia, 361-364.

Gorman, G.C. and Y.J. Kim, 1977. Genotypic evolution in the face of phenotypic conservativeness. Abudefduf (Pomacentridae) from the Atlantic and Pacific sides of Panama. Copeia, **4:** 694-697.

Gorman, C.C. and J. Renzi, 1979. Genetic distance and heterozygosity estimates in electrophoretic studies, effects of sample size. Copeia, **2:** 242-249.

Grant, W.S. and F.M. Utter, 1980. Biochemical genetic variation in walleye pollock, Theragra chalcogramma: population structure in the southeastern Bering Sea and the Gulf of Alaska. Can. J. Fish. Aquat. Sci., **37:** 1093-1100.

Grant, W.S., G.B. Milner, P. Krasnowski and F.M. Utter, 1980. Use of biochemical genetic variants for identification of sockeye salmon (Oncorhynchus nerka) stocks in Cook Inlet, Alaska. Can. J. Fish. Aquat. Sci., **37:** 1236-1247.

Grant, W.S., 1984. Biochemical population genetics of Atlantic herring, Clupea harengus. Copeia, **2:** 357-364.

Grant, W.S. and F.M. Utter, 1984. Biochemical population genetics of Pacific herring (Clupea pallasi). Can. J. Fish. Aquat. Sci., **41:** 856-864.

Grant, W.S., 1986. Biochemical genetic divergence between Atlantic, Clupea harengus and Pacific, C. pallasi herring Copeia., **3:** 714-719.

Grant, W.S., C.I. Zhang, T. Kobayashi and G. Stahl, 1987. Lack of genetic stock discretion in Pacific cod (Gadus macrocephalus). Can. J. Fish. Aquat. Sci., **44:** 490-498.

Graves, J.E., M.J. Curtis, P.A. Oeth and R.S. Waples, 1990. Biochemical genetics of Southern California Basses of the genus Paralabrax: Specific identification of fresh and ethanol-preserved individual eggs and early larvae. Fish. Bull., **88(1):** 59-66.

Gulland, J.A., 1989. Fish populations and their management. J. Fish. Biol., **35A:** 1-9.

Guyomard, R., 1981. Electrophoretic variation in four French populations of domesticated rainbow trout (Salmo gairdneri). Can. J. Genet. Cytol., **23:** 33-47.

Guyomard, R., C. Grevisse, F.X. Oury et P. Davaine, 1984. Evolution de la variabilite genetique inter et intra-population de populations de Salmonides issues de memes pools geniques. Can. J. Fish. Aquat. Sci., **41:** 1024-1029.

Gyllensten, U., N. Ryman and G. Stahl, 1985. Monomorphism of allozymes in perch (Perca fluviatilis L.). Hereditas, **102:** 57-61.

* **Harris, H.,** 1966. Enzyme polymorphisms in man. Proc. Royal Soc. B. 164: 298-310.

* **Harris, H. and D.A. Hopkinson,** 1978. Handbook of enzyme electrophoresis in human genetics. North-Holland Publishing Company. Amsterdam.

Hedgecock, D., 1977. Biochemical genetic markers for broodstock identification in aquaculture. Proc. World Maricult. Soc., **8:** 523-531.

Hedgecock, D., 1986. Genetic analysis of anchovy and sardine populations in the California current system. In: Project Report of Bodega Marine Laboratory, University of California, Davis.

*Heincke, F., 1898. Naturgeschichte des Herings, Teil 1, Abh. dt. Seefisch Ver., Band 2, C XXXVI, 128 pp. text, 223 pp., tables.

Hernandez-Martich, J.D. and M.H. Smith, 1990. Patterns of genetic variation in eastern mosquitofish (Gambusia holbrooki Girard) from the Piedmont and coastal plain of three drainages. Copeia, **3:** 619-630.

Hershberger, W.K., 1992. Genetic variability in rainbow trout populations. Aquaculture, **100 (1-3):** 51-71.

Hodgins, H.O., W.E. Ames and F.M. Utter, 1969. Variants of lactate dehydrogenase isozymes in sera of sock eye salmon (Onchorhynchus nerka). J. Fish. Res. Bd. Canada, **26:** 15-19.

*Hori, S.H., S. Yonezawa, Y. Mochizuki, Y. Sado and T. Kamada, 1975. Evolutionary aspects of animal glucose-6-phosphate dehydrogenase isozymes. In: C.L. Markert (Ed.). Isozymes. Genetics and Evolution, Academic Press, San Francisco, **4:** 839-852.

Hubby, J.L. and R.C. Lewontin, 1966. A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in Drosophila pseudoobscura. Genetics, **54:** 577-594.

Hunter, R.L. and C.L. Markert, 1957. Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. Science, **125:** 1294-1295.

Ihssen, P.E., 1977. Physiological and behavioural genetics and the stock concept for fisheries management, p. 27-30. Proc. fish genetics - fundamentals and implications to fish management, Great Lakes fish comm., Ann Arbor, MI May 10-12.

Ihssen, P.E., D.O. Evans, W.J. Christie, J.A. Reckahn and R.L. Des Jardine, 1981. Life history, morphology and electrophoretic characteristics of five allopatric stocks of lake whitefish (Coregonus clupeaformis) in the Great Lakes region. Can. J. Fish. Aquat. Sci., **38:** 1790-1807.

Ihssen, P.E., H.E. Booke, J.M. Casselman, J.M. McGlade, N.R. Payne and F.M. Utter, 1981a. Stock identification: materials and methods. Can. J. Fish. Aquat. Sci., **38(12):** 1838-1855.

Imhof, M., R. Leary and H.E. Booke, 1980. Population or stock structure of lake whitefish, Coregonus clupeaformis, in northern Lake Michigan as assessed by isozyme electrophoresis. Can. J. Fish. Aquat. Sci., **37:** 783-793.

Jamieson, A., W. de Ligny and G. Naevdal, 1971. Serum esterases in mackerel, Scomber scombrus L. Rapp. P.V. Re'un. Cons. Perm. Int. Explor. Mer., **161:** 109-117.

Jamieson, A., 1974. Genetic tags for marine fish stocks. 91-99 pp. In: F.R. Harden Jones (Ed.). Sea Fisheries Research London. Elek Science, 510 pp.

Jamieson, A. and R.J. Turner, 1980. Muscle protein differences in two eels, Anguilla anguilla (Linnaeus) and Anguilla rostrata (Le Seuer). Biological Journal of the Linnean Society, **13:** 41-45.

Jhingran, A.G., 1984. In: The Fish Genetic Resources of India. National Bureau of Fish Genetic Resources, I.C.A.R., New Delhi. 82 pp.

John, M.A., 1951. Pelagic fish eggs and larvae of Madras Coast. J. Zool. Soc. India, **3:** 38-66.

Johnson, A.G., F.M. Utter and H.O. Hodgins, 1970. Electrophoretic variants of L-alpha-glycerophosphate dehydrogenase in Pacific ocean perch (Sebastodes alutus). J. Fish. Res. Bd. Canada, **27:** 943-945.

Johnson, A.G., F.M. Utter and H.O. Hodgins, 1974. Electrophoretic comparison of five species of pandalid shrimp from the north-eastern Pacific ocean. Fish. Bull., **72:** 799-803.

Johnson, M.S., 1975. Biochemcial systematics of the atherinid genus, Menidia. Copeia, **4:** 662-691.

Jorstad, K.E. and S.A. Pederson, 1986. Discrimination of herring populations in a northern Norwegian fjord: genetic and biological aspects. ICES CM 1986/H: 63.

Jorstad, K.E., D.P.E. King and G. Naevdal, 1991. Population structure of Atlantic herring, Clupea harengus L. J. Fish. Biol., **39:** 43-52.

Kamalakara Rao, A.P., S. Augustine Chellappa, I. Sambasiviah and P.J. Sanjeeva Raj, 1985. Isoenzyme studies in cultivable fishes. Proc. Symp. Coastal Aquaculture, **3:** 984-987.

Kannupandi, T., 1980. Protein patterns during ontogeny of the Xanthid crab, Rhithropanopeus harrisi. Indian J. Mar. Sci., **9:** 127-131.

Kapuscinski, A.R. and D.P. Philipp, 1988. Fisheries genetics : Issues and priorities for research and policy development. Fisheries, **13(6):** 4-10.

Kasinathan R., B.N. Desai and S.N. Durivedi, 1972. Morphometry and electrophoretic studies of Cynoglossus bilineatus (Lac) from Bombay waters. J. Indian Fish. Ass., **2:** 110-115.

Kempf, C. T. and D.K. Underhill, 1974. A serum esterase polymorphism in Fundulus heteroclitus. Copeia, **3:** 792-794.

Kesavan Nair, A.K., K. Balan and P. Prasannakumary, 1973. The fishery of the oil sardine (Sardinella longiceps) during the past 22 years. Indian J. Fish., **20(1):** 223-227.

Koehn, R.K. and D.I. Rasmussen, 1967. Polymorphic and monomorphic serum esterase heterogeneity in catostomid fish populations. Biochem. Genet., **1:** 131-144.

Koehn, R.K., 1970. Functional and evolutionary dynamics of polymorphic esterases in Catostomid fishes. Trans. Am. Fish. Soc., **99(1):** 219-288.

Koehn, R.K. and J.B. Mitton, 1972. Population genetics of marine pelecypods. I. Ecological heterogeneity and evolutionary strategy at an enzyme locus. Am. Nat., **106:** 47-56.

Koehn, R.K., F.J. Turano and J.B. Mitton, 1973. Population genetics in marine pelecypods. II. Genetic differences in microhabitats of Modiolus demissus. Evolution, **27:** 100-105.

Koljonen, M.L., 1986. The enzyme gene variation of ten Finnish rainbow trout strains and the relation between growth rate and mean heterozygosity. Aquaculture, **57:** 253-260.

Kornfield, I.L. and R.K. Koehn, 1975. Genetic variation and specification in new world Cichlids. Evolution, **29(3):** 427-437.

Krieg, F. and R. Guyomard, 1985. Population genetics of French brown trout (Salmo trutta L): large geographical differentiation of wild populations and high similarity of domesticated stocks. Genet. Sel. Evol., **17(2):** 225-242.

Kulkarni, G.K., R. Nagabhushanam and P.K. Joshi, 1980. Electrophoretic separation of protein pattern in different tissues of four marine penaeid prawns in relation to sex. Hydrobiologica, **69:** 25-28.

Kumar, K. and K. Balasubramanyan, 1987. Fishery and biology of oil sardine, Sardinella longiceps from coastal waters of Parangipettai. National Symposium on Research and Development in Marine Fisheries, 16-18 September 1987. Bull. Cent. Mar. Fish. Res. Inst., **44:** 42-45.

Kurian, A., 1977. Effect of gel concentration on the resolution of the muscle myogen of the Bombay duck in PAGE electrophoresis. Indian J. Fish., **24(1-2):** 248-249.

Larkin, P.A., 1972. The stock concept and management of Pacific salmon. H.R. MacMillan lectures in fisheries, Univ. British Columbia. Vancour, B.C. 231 p.

Larkin, P.A., 1981. A perspective on population genetics and salmon management. Can. J. Fish. Aquat. Sci., **38:** 1469-1475.

Lavery, S. and J.B. Shaklee, 1989. Population genetics of two tropical sharks, Carcharhinus tilstoni and C. sorrah in northern Australia. Aust. J. Mar. Freshwater Res., **40:** 541-557.

Lavery, S. and D. Staples, 1990. Use of allozyme electrophoresis for identifying two species of penaeid prawn postlarvae. Aust. J. Mar. Freshwater Res., **41:** 259-266.

Lazarus, S., 1985. On the spawning season and early life history of oil sardine, Sardinella longiceps (Cuv. & Val.) at Vizhinjam. Indian J. Fish., **32(2):** 236-247.

Leberg, P.L., 1990. Influence of genetic variability on population growth: implications for conservation. J. Fish. Biol., **37**: 193-195.

*__Lester, L.J.__, 1979. Population genetics of penaeid shrimp from the Gulf of Mexico. J. Hered., **70**: 175-180.

Lester, L.J., 1983. Developing a selective breeding program for penaeid shrimp mariculture. Aquaculture, **33**: 41-50.

Lewontin, R.C. and J.L. Hubby, 1966. A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of Drosophila pseudoobscura. Genetics, **54**: 595-609.

de Ligny, W., 1969. Serological and biochemical studies on fish populations. Oceanogr. and Mar. Biol., **7**: 411-513.

de Ligny, W. (Ed.), 1971. ICES Special Meeting on the Biochemical and Serological Identification of Fish Stocks, Dublin. 27-28 September 1969. Rapp. P.-V. Reun. Cons. Int. Explor. Mer., **161**: 179 pp.

de Ligny, W., 1972. Blood groups and biochemical polymorphisms in fish. pp. 55-65. In: C. Kovacs and M. Papp (Eds.). XIIth Europ. Conf. Anim. Blood Groups and Biochem. Polymorph., Budapest, 1970. The Hague, Dr. W. Junk, 686 pp.

Liu, L.L., D.W. Foltz and W.B. Stickle, 1991. Genetic population structure of the southern oyster drill, Stramonita (= Thais) haemostoma. Mar. Biol., **111**: 71-79.

Macaranas, J. and Y. Fujio, 1990. Strain differences in cultured fish-isozymes and performance traits as indicators. Aquaculture, **85**: 69-82.

MacDonald, C.M., 1988. Genetic variation, breeding structure and taxonomic status of the gummy shark, Mustelus antarcticus in Southern Australian waters. Aust. J. Mar. Freshwater Res., **39:** 641-648.

MacLean, J.A. and D.O. Evans, 1981. The stock concept, discreteness of fish stocks and fisheries management. Can. J. Fish. Aquat. Sci., **38:** 1889-1898.

Magnusson, K.P. and M.M. Ferguson, 1987. Genetic analysis of four sympatric morphs of Arctic charr, Salvelinus alpinus from Thingvallavatn, Iceland. Env. Biol. Fish., **20(1):** 67-73.

Mahobia, G.P., 1987. Studies on Indian Cichlids. Ph.D. Thesis, Cochin University of Science and Technology, Cochin, Kerala, India.

Mangaly, K.G., 1974. Electrophoretic and biochemical studies on European hake, Merluccius merluccius (L.). Ph.D. Thesis, University of East Anglia, U.K.

Mangaly, G. and A. Jamieson, 1978. Genetic tags applied to the European hake, Merluccius merluccius (L.). Anim. Blood Grps. Biochem. Genet., **9:** 39-48.

Manohar, S.V. and N.K. Velankar, 1973. Separation of muscle proteins of various marine fishes of Bombay by polyacrylamide gel electrophoresis. J. Indian Fish. Association, **1(2):** 8-14.

Markert, C.L. and L. Faulhaber, 1965. Lactate dehydrogenase isozyme patterns of fish. J. Exp. Zool., **159:** 319-332.

Marnell, L.F., R.J. Behnke and F.W. Allendorf, 1987. Genetic identification of cutthroat trout, Salmo clarki in Glacier National Park, Montana. Can. J. Fish. Aquat. Sci., **44:** 1830-1839.

*Marr, J.C., 1957. The problems of defining and recognising subpopulations of fishes. In Contributions to the study of subpopulations of fishes. 1-6 (Cord. J.C. Marr), Spec. Scient. Rep. U.S. Fish. Wildl. Serv., **(208)**: 1-6.

*Marr, J.C. and M. Sprague, 1963. The use of blood groups characteristic in studying populations of fish. Spec. Publs. Int. Common. N.W. Atlant. Fish., **4**: 308-313.

McAndrew, B.J. and K.C. Majumdar, 1983. Tilapia stock identification using electrophoretic markers. Aquaculture, **30**: 249-261.

Menezes, M.R., 1976a. Electrophoretic studies of eye lens and serum proteins of Sardinella fimbriata (Valenciennes) and Sardinella longiceps (Valenciennes). Mahasagar, **8(3&4)**: 117-121.

Menezes, M.R., 1976b. Electrophoretic studies of serum and eye lens proteins of two species of flat fishes. Mahasagar, **8(3&4)**: 151-155.

Menezes, M.R., 1986. An electrophoretic study of the soluble lens proteins from the Indian mackerel, Rastrelliger kanagurta (CUV). Mahasagar, **19(1)**: 69-71.

Menezes, M.R., 1990. Biochemical genetic divergence in three carangids from the Andaman Sea. Curr. Sci., **59(4)**: 209-212.

Menezes, M.R., S. Naik and M. Martins, 1990. Genetic and morphological variations in the Indian mackerel, Rastrelliger kanagurta (Cuvier, 1817) from the Goa region. Proc. Indian Acad. Sci. (Anim. Sci.), **99(6)**: 457-465.

Milkman, R.D. and L.D. Beaty, 1970. Large scale electrophoretic studies of allelic variation in Mytilus edulis. Biol. Bull. Mar. Biol. Lab., Woods Hole. **139**: p.430.

Milner, G.B., D.J. Teel, F.M. Utter and G.A. Winans, 1985. A genetic method of stock identification in mixed populations of Pacific salmon, Onchorhychus spp. Mar. Fish. Rev., **47(1):** 1-8.

Milton, D.A. and J.B. Shaklee, 1987. Biochemical genetics and population structure of blue grenadier, Macruronus novaezelandiae (Hector) (Pisces: Merluccidae), from Australian waters. Aust. J. Mar. Freshwater Res., **38:** 727-742.

Mitton, J.B. and R.K. Koehn, 1975. Genetic organisation and adaptive response of allozymes to ecological variables in Fundulus heteroclitus. Genetics, **79:** 97-111.

Moller, D., 1968. Genetic diversity in spawning cod along the Norwegian coast. Hereditas, **60:** 1-32.

Moller, D., 1970. Genetic diversity in Atlantic salmon and salmon management in relation to genetic factors. In: The International Atlantic Salmon Foundation. Spec. Publ. Ser., **1(1):** 1-30.

Moller, D., 1971. Concepts used in the biochemical and serological identification of fish stocks. In: W. de Ligny (Ed.). Special Meeting on the Biochemical and Serological Identification of Fish Stocks. Rapp. P.-v. Reun. Cons. Int. Explor. Mer., **161:** pp. 7-9.

Mork, J., N. Ryman, G. Stahl, F.M. Utter and G. Sundes, 1985. Genetic variation in Atlantic cod (Gadus morhua) throughout its range. Can. J. Fish. Aquat. Sci., **42:** 1580-1587.

Mulley, J.C. and B.D.H. Latter, 1980. Genetic variation and evolutionary relationships within a group of thirteen species of penaeid prawn. Evolution, **34(5):** 904-916.

Murray, J.H. (Ed.), 1961. Oxford English Dictionary. Oxford Clarendon Press, England.

Muzinic, R. and J.C. Marr., 1960. Population identification. Rep. Sect. 1. In: FAO, Proc. World Sci. Meet. Biology sardines and Relat. species. Vol.1.

Natarajan, V., T. Kannupandi, A.L. Paulpandian and R. Natarajan, 1975. Electrophoretic studies on muscle proteins of some Gobioids of Porto Novo. Bull. Dept. Mar. Sci. Univ. Cochin, **7(3):** 583-589.

Nei, M., 1972. Genetic distance between populations. Am. Nat., **106(999):** 283-292.

Nei, M., 1976. Mathematical models of speciation and genetic distance. In: S. Karlin and E. Nevo (Eds.). Population Genetics and Ecology: 723-765. Academic Press Inc., New York and London.

Nevo, E., 1978. Genetic variation in natural populations: patterns and theory. Theor. Population Biol., **13:** 121.

Northcote, T.G., S.N. Williscroft and H. Tsuyuki, 1970. Meristic and lactate dehydrogenase genotype differences in stream populations of rainbow trout below and above a waterfall. J. Fish. Res. Bd. Canada, **27:** 1987-1995.

Okazaki, T., 1986. Genetic and population structure in masu salmon, Oncorhynchus masou of Japan. Bull. Japan. Soc. Sci. Fish., **52(8):** 1365-1376.

Padhi, B.K. and A.R. Khuda - Bukhsh, 1989. Lactate dehydrogenase in the grass carp, Ctenopharyngodon idella. Curr. Sci., **58(18):** 1041-1044.

* **Pauling, L., H.A. Itano, S.J. Sniger and I.C. Welis,** 1949. Sickle cell anaemia, a molecular disease. Science, **110:** 543-548.

Peterson, G.L. and Z.H. Shehadeh, 1971. Subpopulations of the Hawaiian striped mullet, Mugil cephalus: analysis of variations of nuclear eye-lens protein electropherograms and nuclear eye-lens weights. Mar. Biol., **11:** 52-60.

Philip Samuel, P., 1987. Biochemical genetics of selected commercially important penaeid prawns. Ph.D. Thesis, Cochin University of Science and Technology, Cochin, Kerala, India.

Philipp, D.P., W.F. Childers and G.S. Whitt, 1981. Management implications for different genetic stocks of largemouth bass (Micropterus salmoides) in the United States. Can. J. Fish. Aquat. Sci., **38:** 1715-1723.

Ponniah, A.G., 1988. A study on biochemical genetics on Crassostrea madrasensis of Cochin. In: S. Mahadevan, K.A. Narasimham, K. Satyanarayana Rao, K.M.S. Ameer Hamsa and P. Muthiah (Eds.). National Seminar on Shellfish Resources and Farming. CMFRI Bulletin No.42, pp. 189-192.

Ponnaiah, A.G. 1989. Functional relationship between biochemical genetic polymorphism and physiological variation in aquatic animals. In: P. Das and A.G. Jhingran (Eds.). Fish Genetics in India. Proceedings of the Symposium on Conservation and Management of Fish Genetic Resources of India, held on 11-13 April, 1986. Today and Tomorrow's Printers and Publishers, 24 B/5, Deshbandhu Gupta Road, Karolbagh, New Delhi, 266 pp.

Powell, J.R., 1975. Protein variation in natural populations of animals. In: T. Dobzhansky, M.K. Hecht and W.C. Steere (Eds.). Evolutionary Biology, Vol.8: 79 pp. Plenum, New York.

Prabhu, M.S. and M.H. Dhulkhed, 1967. On the occurrence of small sized oil sardine, Sardinella longiceps Val. Curr. Sci., **36(15):** 410-411.

Prabhu, M.S. and M.H. Dhulkhed, 1972. On two new varieties of the Indian oil sardine, Sardinella longiceps Val. Mahasagar, **5(1):** 27-30.

Present, T.M.C., 1987. Genetic differentiation of disjunct Gulf of California and Pacific outer coast populations of Hypsoblennius jenkinsi. Copeia, **4:** 1010-1024.

Puthran Prathiba, 1984. Electrophoretic studies on Penaeus monodon Fabricus. M.Sc. Dissertation. Cochin University of Science and Technology, Cochin, Kerala, India.

Quinn, T.P., C.C. Wood, L. Margolis, B.E. Riddell and K.D. Hyatt, 1987. Homing in wild sockeye salmon (Oncorhynchus nerka) populations as inferred from differences in parasite prevalence and allozyme allele frequencies. Can. J. Fish. Aquat. Sci., **44:** 1963-1971.

Radovich, J., 1982. The collapse of the California sardine fishery. What have we learned? Cal. COFI. Rep., **23:** 56-78.

Rao, S.N. and M.H. Dhulkhed, 1976. Electrophoretic characteristics of oil sardine (Sardinella longiceps) and mackerel (Rastrelliger kanagurta) eye lens protein. Fish. Technol., **13(1):** 13-15.

Rao, A.P.K., 1981. Electrophoretic studies in some South Indian Mugilidae. A biochemical approach to taxonomy. Ph.D. Thesis, University of Madras, Tamil Nadu, India.

Rao, D.M. and K.S. Rao, 1983. Biometric comparison of Nemipterus japonicus (Bloch) populations along the east coast of India. Proc. Indian Acad. Sci. (Anim. Sci.)., **92(5):** 351-360.

*Raymond, S. and L. Weintraub, 1959. Science, **130**: 711.

Reddy, P.S.R., 1977. Bio-systematic studies in mullets (Family - mugilidae) of Porto Novo (Tamilnadu, S. India). Ph.D. Thesis. Annamalai University.

Redfield, J.A., D. Hedgecock, K. Nelson and J.P. Salini, 1980. Low heterozygosity in tropical marine crustaceans of Australia and the trophic stability hypothesis. Mar. Biol. Lett., **1**: 303-313.

Redfield, J.A. and J.P. Salini, 1980. Techniques of starch gel electrophoresis of penaeid prawn enzymes (Penaeus spp. and Metapenaeus spp.). CSIRO Division of Fisheries and Oceanography Report, 116.

Rees, H., 1967. The chromosome of Salmo salar. Chromosoma, **21**: 472-474.

Reghu, R., 1973. Migration of the juvenile oil sardine, Sardinella longiceps Val., into the backwaters of Cochin. Indian J. Fish., **20(2)**: 655-658.

Rengaswamy, V.S., 1977. Studies on length-frequency of oil sardine at Calicut during 1970-71 to 1973-74. Indian J. Fish., **24(1&2)**: 69-75.

Richardson, B.J., 1982a. Geographical distribution of electrophoretically detected protein variation in Australian commercial fishes. I. Jack mackerel, Trachurus declivis Jenyns. Aust. J. Mar. Freshwater Res., **33**: 917-926.

Richardson, B.J., 1982b. Geographical distribution of electrophoretically detected protein variation in Australian commercial fishes. II. Jackass morwong, Cheilodactylus macropterus Bloch and Schneider. Aust. J. Mar. Freshwater Res., **33**: 917-926.

Richardson, B.J., 1982c. Geographical distribution of electrophoretically detected protein variation in Australian commercial fishes. III. Western king prawn, Penaeus latisulcatus Kishinouye. Aust. J. Mar. Freshwater Res., **33:** 933-937.

Richardson, B.J., 1983. Distribution of protein variation in skipjack tuna (Katsuwonus pelamis) from the central and south-western Pacific. Aust. J. Mar. Freshwater Res., **34:** 231-251.

Richardson, B.J. and G. Habib, 1987. A genetic study of the origins and structuring of the skipjack tuna population exploited by the New Zealand fishery. N.Z.J. Mar. Freshwater Res., **21:** 109-116.

Ricker, W.E., 1972. Heredity and environmental factors affecting certain salmonid populations, p. 19-160. In: R.C. Simon and P.A. Larkin (Eds.). The stock concept in Pacific salmon. H.R. MacMillan Lectures in fisheries, Univ. British Columbia, Vancour. B.C.

Ridgway, G.J., S.W. Sherburne and R.D. Lewis, 1970. Polymorphism in the esterases of Atlantic herring. Trans. Am. Fish. Soc., **99:** 147-151.

Rodino, E. and A. Comparini, 1978. Genetic variability in the European eel, Anguilla anguilla L. In: B. Battaglia and J.A. Beardmore (Eds.). Marine Organisms: Genetics, Ecology and Evolution, pp. 389-423. NATO Conference Series IV. Marine Science, Vol.2. Plenum, New York.

Rosenblatt, R.H. and R.S. Waples, 1986. A genetic comparison of allopatric populations of shore fish species from the Eastern and Central Pacific Ocean: Dispersal or Vicariance? Copeia, **2:** 275-284.

Ryman, N., F.W. Allendorf and G. Stahl, 1979. Reproductive isolation with little genetic divergence in sympatric populations of brown trout (Salmo trutta). Genetics, **92:** 247-262.

Ryman, N. and G. Stahl, 1981. Genetic perspectives of the identification and conservation of Scandinavian stock of fish. Can. J. Fish. Aquat. Sci., **38:** 1562-1575.

Ryman, N., U. Lagercrantz, L. Andersson, R. Chakraborty and R. Rosenberg, 1984. Lack of correspondence between genetic and morphological variability patterns in Atlantic herring (Clupea harengus). Heredity, **53:** 687-704.

Sakaizumi, M., K. Moriwaki and N. Egami, 1983. Allozymic variation and regional differentiation in wild populations of the fish, Oryzias latipes. Copeia, **2:** 311-318.

Salini, J. and J.B. Shaklee, 1988. Genetic structure of barramundi (Lates calcarifer) stocks from Northern Australia. Aust. J. Mar. Freshwater Res., **39:** 317-329.

Sbordoni, V., E. De Matthaeis, M. Cobolli Sbordoni, G. La Rosa and M. Mattoccia, 1986. Bottleneck effects and the depression of genetic variability in hatchery stocks of Penaeus japonicus (Crustacea, Decapoda). Aquaculture, **57:** 239-251.

* **Schmidt, J.,** 1917. Racial investigations. I. Zoarces viviparus L. and the local races of the same. C.R. Trav. Lab. Carlsberg, **13(3):** 277-397.

Seeb, J.E., L.W. Seeb, D.W. Oates and F.M. Utter, 1987. Genetic variation and postglacial dispersal of populations of northern pike (Esox lucius) in North America. Can. J. Fish. Aquat. Sci., **44:** 556-561.

Selander, R.K. and D.W. Kaufman, 1973. Genetic variability and strategies of adaptation in animals. Proc. Natn. Acad. Sci., **70:** 1875.

Selander, R.K., 1976. Genetic variation in natural populations p. 21-45. In: F.J. Ayala (Ed.). Molecular Evolution, Sinauer Associates. Inc., Sunderland. Massachusetts. 277 pp.

Shaklee, J.B., C.S. Tamaru and R.S. Waples, 1982. Speciation and evolution of marine fishes studied by the electrophoretic analysis of proteins. Pac. Sci., **36:** 141-157.

Shaklee, J.B. and J.P. Salini, 1983. Studies suggest multiple stocks of Australian barramundi. Aust. Fish., **42(2):** 36-38.

Shaklee, J.B., 1984. Genetic variation and population structure in the damsel fish, Stegastes fasciolatus throughout the Hawaiian Archipelago. Copeia, **3:** 629-640.

Shaklee, J.B. and P.B. Samollow, 1984. Genetic variation and population structure in a spiny lobster, Panulirus marginatus in the Hawaiian Archipelago. Fish. Bull., **82(4):** 693-702.

Shaklee, J.B. and J.P. Salini, 1985. Genetic variation and population subdivision in Australian barramundi, Lates calcarifer (Bloch). Aust. J. Mar. Freshwater Res., **36:** 203-218.

Shami, S.A. and J.A. Beardmore, 1978. Genetic studies of enzyme variation in the guppy, Poecilia reticulata (Peters). Genetica, **48(1):** 67-73.

Sharp, J.C., K.W. Able, W. Leggett and J.E. Carscadden, 1978. Utility of meristic and morphometric characters for identification of capelin (Mallotus villosus) stocks in Canadian Atlantic waters. J. Fish Res. Bd. Canada, **35:** 124-130.

Shaw, C.R., 1965. Electrophoretic variation in enzymes. Science, **149:** 936-943.

Shaw, C.R. and A.L. Koen, 1968. Starch gel zone electrophoresis of enzymes. In: Chromatographic and Electrophoretic Techniques. J. Smith (Ed.). Second edition. Interscience, New York, **2:** 325-364.

Shaw, C.R. and R. Prasad, 1970. Starch gel electrophoresis of enzymes - A compilation of receipes. Biochem. Genet., **4:** 297-320.

Siciliano, M.J. and R. Shaw, 1976. Separation and visualization of enzymes on gels. In: I. Smith (Ed.). Chromatographic and Electrophoretic Techniques, 4th Edition, Heinemann Medical Books London. **2:** 185-209.

Sick, K., 1961. Haemoglobin polymorphism in fishes. Nature, **192:** 894-896.

Siebenaller, J.F., 1977. Genetic variability in deep sea fishes of the genus Sebastolobus. In: B. Battaglia and J.A. Beardmore (Eds.). Marine Organisms: Genetics, Ecology and Evolution, pp. 95-112. NATO Conference Series IV. Marine Science, Vol.2. Plenum, New York.

Singh, S.M. and R.H. Green, 1984. Excess of allozyme homozygosity in marine molluscs and its possible biological significance. Malacologia, **25:** 569-581.

Skaala, O. and K.E. Jorstad, 1987. Fine-spotted brown trout (Salmo trutta): its phenotypic description and biochemical genetic variation. Can. J. Fish. Aquat. Sci., **44:** 1775-1779.

Smithies, O., 1955. Zone electrophoresis in starch gels: group variation in the serum proteins of normal human adults. Biochem. J. **61:** 629-641.

Smith, I. (Ed.), 1960/1968. In: Chromatographic and Electrophoretic Techniques, 2nd edition. Heinmann Medical Books Ltd., London, **2:** 524 pp.

Smith, P.J., R.I.C.C. Francis and L.J. Paul, 1978. Genetic variation and population structure in the New Zealand snapper. N.Z.J. Mar. Freshwater Res., **12(4):** 343-350.

Smith, P.J. and A. Jamieson, 1978. Enzyme polymorphisms in the Atlantic mackerel, Scomber scombrus L. Comp. Biochem. Physiol., **60B:** 487-489.

Smith, P.J., 1979. Esterase gene frequency and temperate relationships in the New Zealand snapper Chrysophrys auratus. Mar. Biol., **53:** 305-310.

Smith, P.J., G.J. Patchell and P.G. Benson, 1979. Glucose phosphate isomerase and isocitrate dehydrogenase polymorphisms in the hake, Merluccius australis. N.Z.J. Mar. Freshwater Res., **13(4):** 545-547.

Smith, P.J. and A. Jamieson, 1980. Protein variation in the Atlantic mackerel Scomber scombrus. Anim. Blood Grps. Biochem. Genet., **11:** 207-214.

Smith, P.J., J.L. Mckoy and P.J. Machin, 1980. Genetic variation in the rock lobsters, Jasus edwardsii and Jasus novaehollandiae. N.Z.J. Mar. Freshwater Res., **14(1):** 55-63.

Smith, P.J. and D.A. Robertson. 1981. Genetic evidence for two species of sprat (Sprattus) in New Zealand waters. Mar. Biol., **62:** 227-233.

Smith, P.J., R.I.C.C. Francis and A. Jamieson, 1981a. An excess of homozygotes at a serum esterase locus in the Atlantic mackerel, Scomber scombrus. Anim. Blood Grps. Biochem. Genet., **12:** 171-180.

Smith, P.J., G.J. Patchell and P.G. Benson, 1981b. Genetic tags in the New Zealand hoki Macruronus novaezelandiae. Anim. Blood Grps. Biochem. Genet., **12:** 37-45.

Smith, P.J. and Y. Fujio, 1982. Genetic variation in marine teleosts: High variability in habitat specialists and low variability in habitat generalists. Mar. Biol., **69:** 7-20.

Smith, P.J., 1986. Genetic similarity between samples of the orange roughy Hoplostethus atlanticus from the Tasman Sea, South-west Pacific Ocean and North-east Atlantic Ocean. Mar. Biol., **91:** 173-180.

Smith, P.J., 1990. Protein electrophoresis for identification of Australian fish stocks. Aust. J. Mar. Freshwater Res., **41:** 823-833.

Smith, P.J., A. Jamieson and A.J. Birley, 1990. Electrophoretic studies and the stock concept in marine teleosts. J. Cons. Int. Explor. Mer., **47:** 231-245.

Somero, G.N. and M. Soule, 1974. Genetic variation in marine fishes as a test of the niche- variation hypothesis. Nature, **249:** 670-672.

Soule, M., 1976. Allozyme variation: its determinants in space and time. In: F.J. Ayala (Ed.). Molecular Evolution, pp. 60-77. Sinauer Associates, Massachusetts.

Sriraman, K. and P.S. Reddy, 1977. Biochemical studies in planktonic juveniles and adults of Penaeus indicus (Milne Edwards) and Penaeus monodon (Fabricus). Proc. Symp. Warm water zooplankton, NIO.

Stahl, G., 1987. Genetic population structure of Atlantic salmon. In: N.Ryman and F.M. Utter (Eds.). Population Genetics and Fisheries Management. University of Washington Press, Seattle, WA, pp. 121-140.

Stoneking, M., D.J. Wagner and A.C. Hildebrand, 1981. Genetic evidence suggesting subspecific differences between northern and southern populations of brook trout (Salvelinus fontinalis). Copeia, **4:** 810-819.

Sumantadinata, K. and N. Taniguchi, 1982. Biochemical genetic variations in Black Seabrem. Bull. Japan. Soc. Sci. Fish., **48(2):** 143-149.

Suzuki, H. and V.H. Phan, 1990. Electrophoretic study on intraspecific variations and interspecific relationships of marine cat fishes (Siluriformes, Ariidae) of Canane'la (Sao Paulo, Brazil). 2. Isozymes of skeletic muscle. Blom. Inst. Oceanogr., S. Paulo., **38(1):** 43-55.

Thomas, M.M., 1981. Preliminary results of electrophoretic studies on marine prawns, Indian J. Fish., **28(1&2):** 292-294.

Thompson, D., 1985. Genetic identification of trout strains. Aquaculture, **46:** 341-351.

Todd, T.N., G.R. Smith and L.E. Cable, 1981. Environmental and genetic contributions to morphological differentiation in ciscoes (Coregonidae) of the Great Lakes. Can. J. Fish Aquat. Sci., **38:** 59-67.

Tracey, M.L., N.F. Bellet and C.D. Gravem, 1975. Excess allozyme homozygosity and breeding population structure in the mussel. Mytilus Californianus. Mar. Biol, **32:** 303-311.

Tsuyuki, H., E. Roberts and W.E. Vanstone, 1965a. Comparative zone electropherograms of muscle myogens and blood hemoglobins of marine and freshwater vertebrates and their application to biochemical systematics. J. Fish. Res. Bd. Canada, **22(1):** 203-213.

Tsuyuki, H., E. Roberts, W.E. Vanstone and J.R. Markert, 1965b. The species specificity and constancy of muscle myogen and hemoglobin electropherograms of Oncorhynchus. J. Fish. Res. Bd. Canada, **22(1):** 215-217.

Tsuyuki, H. and E. Roberts, 1966. Interspecies relationships within the genus Oncorhynchus based on biochemical systematics. J. Fish. Res. Bd. Canada, **23(1):** 101-107.

Tsuyuki, H., E. Roberts, R.H. Lowes, W. Hadaway and S.J. Westrheim, 1968. Contribution of protein electrophoresis to rockfish (Scorpaenidae) systematics. J. Fish. Res. Bd. Canada, **25(11):** 2477-2501.

Tsuyuki, H., E. Roberts and E.A. Best, 1969. Serum transferrin systems and the haemoglobins of the Pacific halibut (Hippoglossus stenolepis). J. Fish. Res. Bd. Canada, **26:** 2351-2362.

Uthe, J.F. and R.A. Ryder, 1970. Regional variation in muscle myogen polymorphism in walleye (Stizostedion vitreum vitreum) as related to morphology. J. Fish. Res. Bd. Canada, **27:** 923-927.

Utter, F.M., 1969. "Biochemical polymorphism in the Pacific hake (Merluccius productus): a - esterase polymorphism in vitreous fluids, b - lactate dehydrogenase isozymes, c - transferrin variants". Ph.D. thesis. University of California, Davis, 60 pp.

Utter, F.M., C.J. Stormont and H.O. Hodgins, 1970. Esterase polymorphism in vitreous fluid of Pacific hake, Merluccius productus. Anim. Blood Grps. Biochem. Genet., **1:** 69-82.

Utter, F.M. and H.O. Hodgins, 1972. Biochemical genetic variation at six loci in four stocks of rainbow trout. Trans. Am. Fish. Soc., **3:** 494-502.

Utter, F.M., F.W. Allendorf and H.O. Hodgins, 1973. Genetic variability and relationships in Pacific salmon and related trout, based on protein variations. Sys. Zool., **22(3):** 257-270.

Utter, F.M., H.O. Hodgins and F.W. Allendorf, 1974. Biochemical genetic studies of fishes: Potentialities and limitations. In: Malins, D. (Ed.). Biochemical and Biophysical Perspective in Marine Biology, Vol.1, p. 213-237. Academic Press, San Francisco.

Utter, F.M., 1981. Biological criteria for definition of species and distinct intraspecific populations of anadromous salmonids under the U.S. Endangered Species Act of 1973. Can. J. Fish. Aquat. Sci., **38(12):** 1626-1635.

Utter, F.M., 1987. Protein electrophoresis and stock identification in fishes. In: H.E. Kumpf., R.N. Vaught, C.B. Grimes, A.G. Johnson and E.L. Nakamura (Eds.). Proceedings of the Stock Identification Workshop. NOAA Technical Memorandum NMFS - SEFC - 199. Nov. 5-7, 1985. Panama City Beach, Florida, pp. 63-104.

Utter, F.M., P. Aebersold and G. Winans, 1987. Interpreting genetic variation detected by electrophoresis. In: N. Ryman and F.M. Utter (Eds.). Population Genetics and Fishery Management. Washington Sea Grant Program, University of Washington Press, Seattle and London, pp. 21-45.

Utter, F.M., G.B. Milner, G. Stahl and D.J. Teel, 1989. Genetic population structure of chinook salmon, Oncorhynchus tshawytscha in the Pacific northwest. Fish. Bull., **87:** 239-264.

Utter, F.M., R.S. Waples and D.J. Teel, 1992. Genetic isolation of previously indistinguishable chinook salmon populations of the Snake and Klamath rivers: Limitations of negative data. Fish. Bull., **90(4):** 770-777.

Utter, F.M. and N. Ryman, 1993. Genetic markers and mixed stock fisheries. Fisheries, **18(8):** 11-21.

Valentine, J.W. and F.J. Ayala, 1974. Genetic variation in Frieleia halli, a deep sea brachipod. Deep Sea. Res., **22:** 37.

Valentine, J.W., 1976. Genetic strategies of adaptation. In: F.J. Ayala (Ed.). Molecular Evolution, pp. 78-94. Sinauer Associates, Massachusetts.

*Vawter, A.T., R.H. Rosenblatt and G.C. Gorman, 1980. Genetic divergence among fishes of the eastern Pacific and Caribbean : support for the molecular clock. Evolution, **34**: 705-711.

Velappan Nair, R., 1959. Notes on the spawning habits and early life history of the oil sardine, Sardinella longiceps (Cuv. & Val.). Indian J. Fish., **6(2)**: 342-349.

Verspoor, E., 1988. Reduced genetic variability in first generation hatchery populations of Atlantic salmon (Salmo salar). Can. J. Fish. Aquat. Sci., **45**: 1686-1690.

Vijayakumar, S., 1992. Studies on biochemical genetics of the grey mullet, Mugil cephalus (Linnaeus). Ph.D. Thesis, Cochin University of Science and Technology, Cochin, Kerala India. 113 pp.

Waples, R.S., G.A. Winans, F.M. Utter and C. Mahnken, 1990. Genetic approach to the management of Pacific salmon. Fisheries, **15(5)**: 19-25.

Ward, R.D., 1977. Relationship between enzyme heterozygosity and quaternary structure. Biochem. Genet., **15**: 123.

Ward, R.D. and J.A. Beardmore, 1977. Protein in the plaice, Pleuronectes platessa L. Genet. Res., **36**: 45-62.

Ward, R.D. and R.A. Galleguillose, 1977. Protein variation in the plaice, the dab and the flounder and their genetic relationships. In: B. Battaglia and J.A. Beardmore (Eds.). Marine Organisms: Genetics, Ecology and Evolution, NATO Conference Series. IV. Marine Science, Plenum, New York, **2**: 71-93.

Ward, R.D., 1978. Subunit size of enzymes and genetic heterozygosity in vertebrates. Biochem. Genet., **16(7/8):** 799-810.

Wehrhahn, C.F. and R. Powell, 1987. Electrophoretic variation, regional differences, and gene flow in the coho salmon (Oncorhynchus kisutch) of southern British Columbia. Can. J. Fish. Aquat. Sci., **44:** 822-831.

Whitt, G.S. and J.J. Horowitz, 1970. Evolution of a retinal specific lactate dehydrogenase isozyme in teleost. Experientia, **26:** 1302-1304.

Williams, G.C., R.K. Koehn and J.B. Mitton, 1973. Genetic differentiation without isolation in the American eel, Anguilla rostrata. Evolution, **27:** 192-204.

Williscroft, S.N. and H. Tsuyuki, 1970. Lactate dehydrogenase systems of rainbow trout-evidence of polymorphism in liver and additional subunits in gills. J. Fish. Res. Bd. Canada, **27:** 1563-1567.

Winans, G.A., 1980. Geographic variation in the milkfish, Chanos chanos I. Biochemical evidence. Evolution, **34(3):** 558-574.

Wishard, L.N., J.E. Seeb and F.M. Utter, 1980. Biochemical genetic characteristic of native trout populations of Owyhee County, Idaho. Final Report, Bureau of land management, Boise district Office, Pacific Fisheries Research, 509, East 12th // 10, Olympia, Washington, 98501.

Withler, R.E., 1985. Ldh-4 allozyme variability in North American sockeye salmon (Onchorhynchus nerka) populations. Can. J. Zool., **63:** 2924-2932.

Wright, J.E. and L.M. Atherton, 1970. Polymorphisms for LDH and transferrin loci in brook trout populations. Trans. Am. Fish. Soc., **1:** 179-192.

Yamauchi, T. and E. Goldberg, 1973. Glucose-6-phosphate dehydrogenase from brook, lake and splake trout: An isozymic and immunological study. Biochem. Genet., **10:** 121.

Zouros, E., 1976. Hybrid molecules and the superiority of the heterozygote. Nature, **262:** 227.

Zouros, E. and D.W. Foltz, 1984. Possible explanations of heterozygote deficiency in bivalve molluscs. Malacologia, **25:** 583-591.

---

* Not referred original